Journal of Helminthology (1980) **54**, 183 – 190

The distribution of microfilariae in the skin of Guatemalan onchocerciasis patients: an evaluation of diagnostic potentials

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ABSTRACT

Quantitative examinations for the distribution and density of microfilariae were carried out on male Guatemalan onchocerciasis patients by taking 6 or 13 skin biopsies each from 74 subjects. We found that microfilariae were distributed most frequently on the iliac crest and secondarily in the scapular region; higher detection rates and greater microfilarial densities were seen in these areas. From these results we recommend the taking of biopsies from both of these anatomical regions so as to avoid false negative cases. The infected patients were classified into 3 groups (light, moderate and heavy infections) on the basis of the numbers of negative skin snips out of the 13 biopsies taken. In heavy infections, a higher densities of microfilariae were detected in the head and neck regions, as compared with the densities in the lower extremities (calf). The high concentration of microfilariae in these regions is of importance in relation to the development of eye lesions and/or transmission. Of patients who had microfilariae in the anterior segment of the eye some had negative skin biopsies from the outer canthus, retroauricular region and neck.

Microfilarial distribution in the skin of onchocerciasis patients is closely related not only to the clinical manifestations, such as skin changes, ocular lesions and blindness, but also to the location of onchocercal nodules in the bodies of patients (Kershal *et al.*, 1954). However, the microfilarial distribution varies with the geographic distribution of the disease as mentioned by the World Health Organization Expert Committee (1976). For this reason, several investigations have been carried out up to the present time in different endemic areas of onchocerciasis in the world. In Mexico, Mazzotti (1951) carried out qualitative examinations on microfilarial distribution by taking skin biopsies from various areas of the body. He suggested that the microfilariae had a tendency to be distributed irregularly throughout the entire skin of the upper half of the body and the pelvic girdle. In Guatemala, De Leon and Duke (1966) described the microfilarial distribution in the skin of patients based on the numbers of microfilariae ingested by *Simulium ochraceum*. They reported that the microfilariae were most abundant in all areas of skin from the face down to the buttocks; while very much smaller numbers were found in the legs. In their case, the results are influenced by factors dependent on the vector. A detailed understanding of the distribution of microfilariae in the skin of patients in each endemic region of the disease is important for the purpose of diagnosis or epidemiological surveys. Such information will indicate the most suitable biopsy site in an onchocerciasis endemic area. Thus, we have investigated the distribution and density of microfilariae by taking numerous skin biopsies from various areas of the body from patients in Guatemala.

MATERIALS AND METHODS

To investigate microfilarial distribution in onchocerciasis patients from Guatemala, 13 skin snips each from 52 subjects were taken from various areas of the body (Fig. 1).

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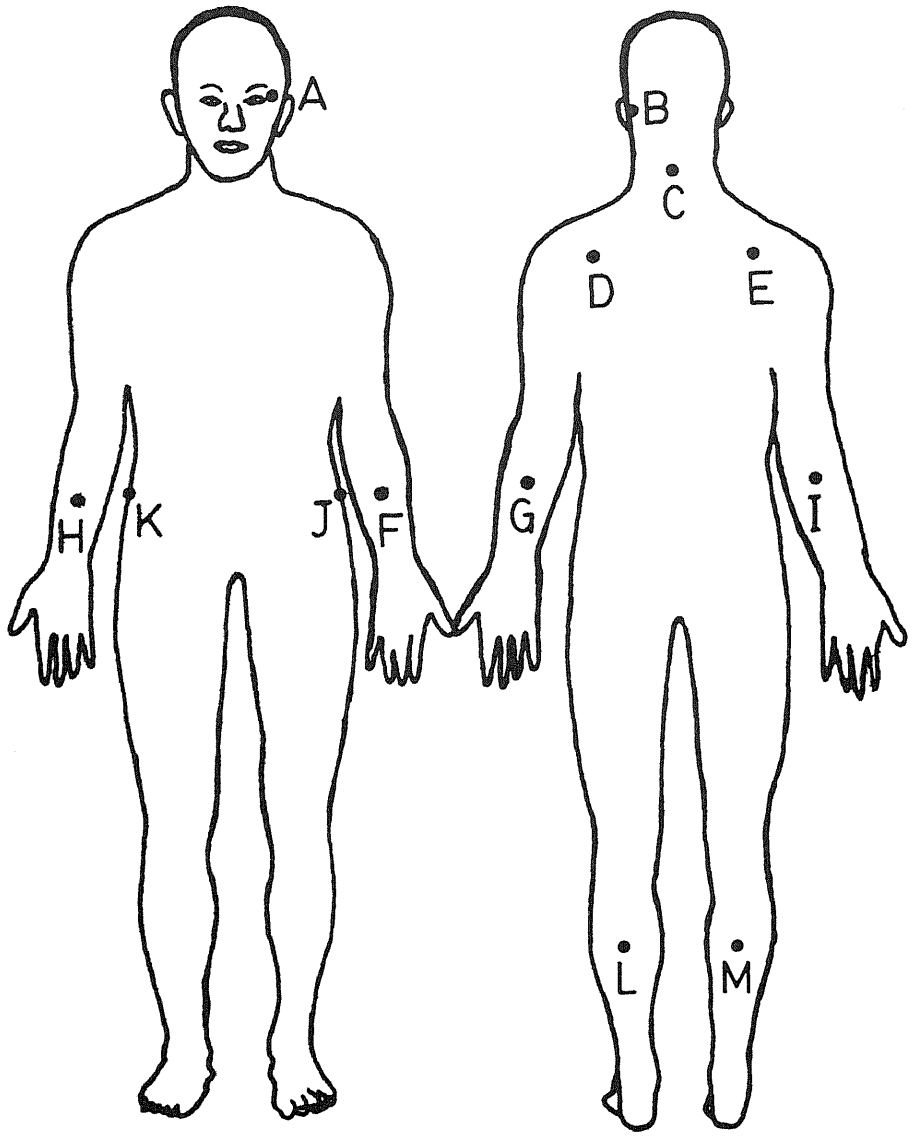

FIG. 1. Sites of skin biopsies taken by Holth type corneoscleral punch.
Key: A = Left outer canthus; B = Left retro-auricular region; C = Back of neck; D = Left scapula; E = Right scapula; F = Left anteriority of upper extremities; G = Left posteriority of upper extremities; H = Right anteriority of upper extremities; I = Right posteriority of upper extremities; J = Left iliac crest; K = Right iliac crest; L = Left calf; M = Right calf.

Only 6 biopsies each were taken from 22 subjects from a highly endemic area. All of the examinees in this study had been detected in an earlier preliminary examination. For taking skin snips the German-made Holth type corneoscleral punch was used. Skin snips taken were put into a drop of 0.9% physiological saline on a glass slide and incubated at room temperature for one hour. Unstained microfilariae emerging from skin snips were

counted immediately at 40× magnification under a compound microscope. The number of microfilariae obtained per 1 mm^2 was used as the expression of microfilarial density in this study. Only male subjects, aged 13 or over, were examined. The examinations were carried out during September to December, 1977 at Aldea Hoja Blanca, Departamento de Huehuetenango, Finca San Rafael Sumatan, Departamento de Chimaltenango, Finca Monte de Oro, Departamento de Suchitepéquez and Población de Palin, Departamento de Escuintla, Guatemala.

Eye lesions and microfilariae in the anterior chamber (segment) of the patients were examined using a Kowa portable slit lamp, a Nikon photo slit-lamp and a direct ophthalmoscope.

The results obtained were analysed statistically by χ^2 test.

RESULTS

By taking 6 or 13 skin snips each from the subjects examined, the highest detection rate for microfilariae was recognized in biopsies from the iliac crest of patients; skin biopsies from the scapula also showed a relatively high rate (Table 1). However, no statistical differences in detection rates between the two biopsy sites mentioned above were recognized ($p>0.05$). In this examination, the biopsies without microfilariae (negative biopsies) occurred most rarely in the iliac crest (5.4% in left, 13.5% in right), next in the scapula (14.9% in left, 19.2% in right), then in the neck region (24.3%) and 33.8% to 55.8% were negative for microfilariae in other biopsy sites.

For microfilarial densities, both the left and right iliac crest showed the highest density of microfilariae; the density in the scapular region was higher than that from the other biopsy sites (Table 2). No highly significant differences, however, were found in the microfilarial densities between scapula and iliac crest of the patients in each location where the examinees came from. In those subjects from which 13 biopsies were taken, infected patients were classified into three groups according to the number of negative biopsies—that is, those with: (1) 6 to 12 negatives (light infection), (2) 3 to 5 negatives (moderate infection), (3) 0 to 2 negatives (heavy infection). In comparing microfilarial densities in the upper (F, G, H and I) and the lower (L and M) extremities, the former had a statistically higher density than the latter in heavy infections, as shown in Table 3 ($p<0.001$). The density in the outer canthus, retro-auricular region and neck showed a rapid rise in heavy infections as compared with those in light and moderate infections. No remarkable unilateral distribution of the microfilariae was found in the present patients.

The density of microfilariae in the outer canthus, retro-auricular region and neck would be most important for estimating microfilarial densities in the head region, especially with relation to eye lesions and blindness. To address this point, 22 subjects, all of whom had a moderate or severe eye lesion which was probably caused by onchocerciasis, were examined at Población Palin (Table 4). These residents had high microfilarial densities in the regions mentioned above; no statistical differences were recognized in the densities between B and D, between B and J, and between D and J ($p>0.05$). The relation of the distribution and density of microfilariae to skin lesions and to eye changes will be reported on elsewhere.

In the present examination, 17 (32.7%) of the 52 patients having 13 skin biopsies had typical onchocercal nodules. Of these nodules, 7 (41.2%) were recognized on the head, 8 (47.0%) on the torso and 2 (11.8%) were found on both the head and torso. No significant correlations were recognized between the location of nodules and the microfilarial densities in each biopsy from various areas of the patients.

TABLE 1

Detection rate for microfilariae in each biopsy area by taking 13 or 6 skin snips each from 74 subjects who were positive for microfilariae in at least one biopsy

Biopsy area	Locations where the examinees came from								Total no. positive biopsies/Total no. examined (%)	
	Hoja Blanca		San Rafael Sumatan		Monte de Oro		Palin			
	No. positive biopsies n* = 5	%	No. positive biopsies n = 35	%	No. positive biopsies n = 12	%	No. positive biopsies n = 22	%		
A	3	60.0	14	40.0	5	41.7	16	72.7	38/74	(52.8)
B	2	40.0	21	60.0	4	33.3	19	86.4	46/74	(63.9)
C	2	40.0	27	77.1	7	58.3	20	90.0	56/74	(77.8)
D	3	60.0	29	82.9	9	75.0	22	100.0	63/74	(87.5)
E	3	60.0	29	82.9	10	83.3	—	—	42/52	(81.8)
F	2	40.0	17	48.6	4	33.3	—	—	23/52	(44.2)
G	2	40.0	23	65.7	5	41.7	—	—	30/52	(57.7)
H	2	40.0	23	65.7	5	41.7	—	—	30/52	(57.7)
I	1	20.0	26	74.3	7	58.3	—	—	34/52	(65.4)
J	4	80.0	32	91.4	12	100.0	22	100.0	70/74	(97.2)
K	3	60.0	31	88.6	11	91.7	—	—	45/52	(86.5)
L	3	60.0	23	65.7	9	75.0	14	63.6	49/74	(68.1)
M	2	40.0	22	62.9	4	33.3	—	—	28/52	(53.9)

Key: A = Left outer canthus; B = Left retro-auricular region; C = Back of neck; D = Left scapula; E = Right scapula; F = Left anteriority of upper extremities; G = Left posteriority of upper extremities; H = Right anteriority of upper extremities; I = Right posteriority of upper extremities; J = Left iliac crest; K = Right iliac crest; L = Left calf; M = Right calf

*Number of subjects examined in each location

TABLE 2

Microfilarial densities per 1 mm² skin snip in each biopsy area by taking 13 skin snips each from the 52 subjects (keys to letters as in Table 1)

	Locations where the examinees came from		
	Hoja Blanca	San Rafael Sumatan	Monte de Oro
Biopsy area	Density (Gm*) n† = 5	Density (Gm) n = 35	Density (Gm) n = 12
A	0.17	0.20	0.12
B	0.06	0.44	0.11
C	0.24	1.06	0.66
D	0.84	2.22	1.60
E	0.72	3.25	2.15
F	0.16	0.55	0.21
G	0.10	0.86	0.42
H	0.10	0.70	0.31
I	0.17	1.14	0.50
J	2.68	5.00	5.72
K	1.22	4.38	3.98
L	0.39	0.43	0.38
M	0.20	0.30	0.10

* Geometric mean (William's mean)
† Number of subjects examined in each location

TABLE 3

Distribution of microfilariae in the patients who are divided by the number of negative biopsies (without microfilariae) into light, moderate and heavy infections (keys to letters as in Table 1)

	Intensity of infection		
	Light infection	Moderate infection	Heavy infection
Biopsy area	Density (Gm*) n† = 17	Density (Gm) n = 17	Density (Gm) n = 18
A	0.04	0.05	0.79
B	0.02	0.20	1.36
C	0.09	0.95	3.69
D	0.28	3.44	5.34
E	0.38	3.82	10.60
F	0.02	0.23	2.88
G	0.02	0.75	3.14
H	0.03	0.36	3.14
I	0.01	0.77	6.64
J	1.31	9.85	8.33
K	0.75	7.41	8.74
L	0.22	0.51	0.59
M	0.05	0.36	0.40

* Geometric mean density of microfilariae per 1 mm² skin snip
† Number of subjects examined

TABLE 4

Microfilarial densities per 1 mm^2 skin snip in each biopsy area from male patients from Población Palin all of whom had eye lesions which might be caused by onchocerciasis

Patient no.	Age	No. of microfilariae in anterior chamber	Biopsy area*					
			A	B	C	D	J	L
1	59	10	17.0	33.0	57.4	36.1	13.3	0.4
2	13	5	5.5	1.3	0.3	0.6	0.3	0.5
3	72	16	50.6	73.2	97.4	13.8	9.9	0
4	38	0	3.5	1.8	5.2	4.7	24.0	4.4
5	59	0	0	2.0	6.3	4.6	0.4	0.6
6	47	1	11.8	21.4	24.3	27.3	131.4	0.7
7	27	0	0	0	1.5	0.9	1.0	0
8	41	6	71.5	15.2	28.5	75.3	29.5	15.0
9	40	0	0	12.9	0	9.9	6.6	0
10	50	5	22.0	16.8	3.4	2.0	6.4	0
11	41	0	0	1.6	1.1	0.9	1.7	0
12	29	1	9.0	0	2.0	12.8	10.8	1.5
13	13	6	27.2	23.2	136.3	66.5	211.9	18.9
14	60	2	1.8	106.5	56.5	53.3	32.0	1.5
15	42	4	0	4.5	0	24.8	42.2	0.3
16	48	5†	46.3	96.2	221.6	4.5	24.1	4.4
17	59	5†	17.5	4.4	48.1	8.5	17.1	0.2
18	24	0	1.3	0.4	4.5	7.1	87.8	1.8
19	25	1	35.2	3.0	5.5	4.9	1.5	0
20	52	16	82.5	52.5	9.3	28.6	23.0	6.0
21	14	0	1.7	0	0.9	0.9	1.8	0
22	27	1	0	4.7	3.1	3.7	37.0	0

*Key: A = Left outer canthus; B = Left retro-auricular region; C = Back of neck; D = Left scapula; J = Left iliac crest; L = Left calf

†More than five

DISCUSSION

From the diagnostic point of view, it is important to determine the detection rate for onchocerciasis patients based on the distribution and density of microfilariae in the skin. Such information would indicate the biopsy site most likely to contain microfilariae in different endemic areas of the disease. Mazzotti (1951), in Mexico, showed that microfilariae have been most frequently found at the level of the shoulders when skin biopsies were taken from the cheeks, shoulders, forearms, hands, costal regions, thighs and feet. From these results he suggested that microfilariae have a tendency to be distributed irregularly throughout the entire skin of the upper half of the body and the pelvic girdle and were rarely found in the skin of the lower extremities. According to the World Health Organization Expert Committee (1976), the optimum site for biopsy will vary depending on the geographical strain of the parasite. In Africa, therefore, the preferred site is below the iliac crest, whereas in Mexico the preferred site is behind the shoulder. In the present onchocerciasis patients from Guatemala, the iliac crest had a tendency to be the site of the highest detection rate and the greatest microfilarial density as compared with other skin biopsy sites. Between the scapula and iliac crest, however, no highly significant differences were found in both the rate and density. From the results obtained in this study, it is recommended that 2 biopsies should be taken from the iliac crest and/or the scapular regions of male Guatemalan patients, so as to avoid false negative cases. In a low endemic area (Hoja Blanca), however, the differences in detection rates and microfilarial densities were not so remarkable among the 13 biopsy sites because of the irregular distribution of small number of microfilariae in the skin throughout the body.

In this investigation the 52 subjects from whom were taken 13 biopsies were classified into 3 groups. Comparing the microfilarial densities in these groups, remarkable differences between the head regions and the lower extremities were recognized in the case of heavy infection, indicating a higher density in the upper parts of the body. In light and moderate infections, microfilarial densities showed no great differences in biopsies taken from the upper and lower parts of the body. De Leon and Duke (1966) quantified the numbers of microfilariae which were taken in by *Simulium ochraceum* from 32 areas of the body in Guatemala. They showed that microfilariae were most abundant in all areas of skin from the face to the buttocks, while smaller numbers were recognized in the legs. They also suggested that this concentration in the upper parts of the body in Guatemala is the reverse of the normal distribution seen in West African patients by Kershaw *et al.* (1954). Their results appeared to indicate a relatively higher concentration of microfilariae because of the possible existence of some attractant factor in the saliva of black-flies (Strong *et al.*, 1934; Dalmat, 1955; De Leon and Duke, 1966; Duke, 1970). From the point of view of transmission, the distribution and concentration in the head and neck regions and also in the upper extremities of the body would be very important because the main vector, *Simulium ochraceum,* predominantly bites the upper parts of the body in Guatemala (De Leon and Duke, 1966).

In the distribution and density of the microfilariae in 22 residents from a high endemic area (Población de Palin), 2 biopsies each taken from the left scapula and left iliac crest were positive for microfilariae in all of the subjects examined. In the other biopsy sites, one or more negative skin snips were recognized. In relation to eye lesions and/or blindness the concentration of microfilariae near the outer canthus is one of the most important measures for estimating the risk factor (Fuglsang and Anderson, 1977). In the present study, however, 6 (27.3%) of the 22 subjects had negative skin biopsies in the outer canthus of the eye of the present patients, although all of them had a moderate or severe eye lesion with 0 to 16 microfilariae in their anterior chamber. The densities of microfilariae in the outer canthus of these subjects varied from 0 to 82.5 per 1 mm^2 skin snip. The densities in the retro-auricular regions and neck regions may also be used to estimate the possibility of ocular lesions. No marked differences in the microfilarial densities were found between the lower and the higher age group of the positive patients with microfilariae in the anterior chamber.

In 2 groups which had nodules on the head or on the torso, the distribution and density of the microfilariae was examined. However, no marked correlation between the location of nodules and the distribution or the density of the microfilariae was recognized in the present patients in Guatemala. In West Africa, microfilariae in a light infection were found in a higher concentration in the same quarter as the nodule, while a high concentration in the lower extremities occurred irrespective of the position of the nodules though the nodules are predominantly on the legs and pelvis. The results described above pertain to present-day conditions. Therefore, the relation between the distribution of microfilariae and the nodules may be modified by the national denodulectomy campaign which has been carried out for a long time in Guatemala.

ACKNOWLEDGEMENTS

This study was supported by the Ministry of Public Health, Republic of Guatemala, and by the Japan International Cooperation Agency (JICA) (GJCRCPO-MENSAP series no. 12). We wish to thank Drs. L. R. Ash and S. Hayashi for critical reading and valuable comments; Drs. J. J. Castillo O., H. Figueroa M. and H. Takahashi for their encouragement thoughout this study; and to express our appreciation to our collaborators in Servicio Nacional de Erradicación de la Malaria, Guatemala. We are also indebted to Messrs. J. O. Ochoa and P. A. Molina who kindly helped our field works throughout the investigation.

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Accepted 26 January, 1980.