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Evaluation of skin biopsies from different body regions of onchocerciasis patients in Central Nigeria

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Summary

The diagnostic potential of skin snips from different body regions was evaluated in 97 onchocerciasis patients of Central Nigeria. Biopsies from the iliac crest had the greatest diagnostic potential followed by those of the calf and shoulder with 95% of *Onchocerca volvulus* carriers being diagnosed from these sites. However, 23.7% of microfilarial carriers in the skin of the outer canthus, shoulder, calf and ankle were negative in the iliac crest. The probability of diagnosing onchocerciasis in microfilarial positives from the 5 sites was, 0.76 for the iliac crest; 0.63 for the calf; 0.31 for the shoulder; 0.27 for the ankle; and 0.08 for the outer canthus. The concentrations of microfilariae in skin snips were significantly associated with the probability of a positive diagnosis especially with regard to those of the shoulder, iliac crest and calf.

Key words: *Onchocerca volvulus*; microfilariae; skin snips; evaluation.

Introduction

The demonstration of the microfilariae of *O. volvulus* in skin biopsies provides the most convincing evidence of the disease in patients. The World Health Organization Expert Committee on onchocerciasis (1966) observed that the distribution of the microfilariae of *O. volvulus* in patients varies from one

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disease focus to the other. High densities of microfilariae are usually found in those body regions where the local *Simulium* vectors attack humans for blood meal (Kershaw et al., 1954; Duke and Beesley, 1958; De Leon and Duke, 1966; WHO, 1966; Anderson et al., 1973; Buck, 1974; Kawabata et al., 1980). However, due to the discrepant epidemiology and clinical pathology of onchocerciasis in the local populations of West Africa (Duke, 1976), it has become necessary to study the distribution of microfilariae from different anatomical sites in onchocerciasis patients for accurate diagnosis of the disease in the sub-region.

Materials and Methods

The investigation was carried out between June and October 1986. The study areas (Kudaru, 10°33'N, 8°28'E; Zendi, 9°55'N, 9°55'N, 9°15'E) which are located in the northern Guinea savanna have been described (Crosskey, 1954; Ufomadu et al., 1986).

Ninety-seven patients (Kudaru, 20; Zendi, 77), 16 to 70 years of age were examined for onchocercal nodules and other manifestations of onchocerciasis. Skin snips were taken from the area of the outer canthus (A), shoulder (B), iliac crest (C), lower calf (D), and ankle (E) using a Holth corneo-scleral punch with 2 mm bite. Skin snips were placed in Tyrode solution in flat-bottom microtitre wells separated by anatomical site and patient as described by Barbiero and Trips (1984). The microtitre wells were sealed with adhesive tape for incubation over night.

Each microtitre well was examined directly using a WILD MP 511 inverted microscope at low magnifications. After counting, snips were removed by means of forceps, rinsed in distilled water, blotted between filter papers, and weighed on an electric analytical balance. Microfilarial densities were expressed per milligramme skin. The results were subjected to Chi-square analysis for significant variations.

Results

Frequency of microfilaria positive biopsies

The average weight (\pm standard deviation) of 485 skin biopsies from 5 anatomical sites of 97 patients was 2.0 mg (\pm 0.6). Table 1 shows the frequency distribution of *O. volvulus* microfilariae by anatomical site.

Twenty-three (23.7%) cases with positive biopsies of the lower calf, shoulder, ankle and outer canthus were negative in the iliac crest. Of the 97 patients, 93 (95.9%) were diagnosed from the shoulder, iliac crest and calf regions. Skin snips from the iliac crest had the highest diagnostic yield ($n = 74$, 76.3%); followed by the lower calf ($n = 16$, 16.5%); shoulder ($n = 3$, 3.1%); ankle ($n = 2$, 1.1%); and outer canthus ($n = 2$, 2.1%). The probability of correctly diagnosing onchocerciasis in microfilarial carriers from the 5 sites was 0.76 for the iliac crest; 0.63%, calf; 0.31, shoulder; 0.27, ankle; and 0.08, outer canthus. In many instances, biopsies from 2 or more sites revealed microfilariae simultaneously.

Table 1. Microfilariae in skin biopsies of 97 (= 100%) onchocerciasis patients

Anatomical sites*	Frequency of positive biopsies	
	n	%
A	2	2.1
B	2	2.1
C	19	19.6
D	10	10.3
E	2	2.1
AB	1	1.0
AC	1	1.0
AD	1	1.0
AE	0	0
BC	4	4.1
BD	1	1.0
BE	0	0
CD	17	17.5
CE	4	4.1
DE	4	4.1
ABCDE	1	1.0
ABCD	1	1.0
ABC	1	1.0
BCDE	8	8.3
BCD	11	11.3
CDE	7	7.2

*A = outer canthus; B = scapula; C = iliac crest; D = lower calf; E = ankle

Distribution of microfilarial densities

Table 2 shows the proportion of positive biopsies and microfilarial densities (Mfds) in the 5 body sites. Statistically significant differences ($p < 0.05$) were observed between mean Mfds and anatomical locations.

Localization of nodules in patients

Most nodules were situated in the lower limbs and lumber/abdominal regions (Table 2) where peak mean Mfds were found, the lower limbs being the sites often attacked by *S. damnosum* s. l. in the savanna of West Africa (Renz and Wenk, 1983).

Discussion

The results of this study in the Guinea savanna of Nigeria demonstrate the diagnostic usefulness of simultaneously sampling different parts of the body. The merits of this method of sampling in epidemiological study, and in the

Table 2. Localization of nodules and microfilarial densities in relation to positive biopsies

Anatomical site*	No. (%) positive biopsies	Microfilarial density**	Nodules*** (%)
A	8 (4.0)	6.2	7 (6.3)
B	30 (15.1)	46.2	27 (24.1)
C	74 (37.2)	95.6	37 (33.0)
D	61 (30.7)	81.3	36 (32.1)
E	26 (13.1)	50.4	5 (4.5)
Total	199		112

* as in Table 1

** Geometric mean (microfilarial density per mg/tissue)

*** Localization: A = cephalic; B = thorax and upper limbs; C = lumbar and abdominal; D = thigh, knee and sheen; E = ankle region

determination of microfilarial densities and the changes induced by chemotherapy have been pointed out and described in other endemic foci of onchocerciasis (Buck, 1974; Awadzi et al., 1980; Kawabata et al., 1980; Greene et al., 1985). As has been demonstrated, the concentrations of microfilariae in skin snips were significantly associated with the probability of a positive diagnosis especially with regard to biopsies from the iliac crest, the calf and shoulder regions. These correlated with the anatomical distribution of nodules in patients, a fact which has been recognized in West African onchocerciasis (Kershaw et al., 1954; Duke, 1957).

This study showed that the result from a single skin biopsy can be misleading (Duke et al., 1967), and confirms previous reports (Blacklock, 1926; Barbiero and Trips, 1984) that less than 80% of West African patients are diagnosed when skin snips are taken only from the iliac region, the site recommended for biopsy in Africa (WHO, 1966, 1976).

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