

ONCHOCERCOMAS IN GUATEMALA, WITH SPECIAL REFERENCE TO APPEARANCE OF NEW NODULES AND PARASITE CONTENT*

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Abstract. To determine the rate of appearance of new nodules during a specified period, a follow-up study on onchocercomas that had been removed was carried out in San Vicente Pacaya, Guatemala, between June 1976 and April 1977. Incidence rate, or rate of nodules appearing in initially negative subjects, was studied in relation to the degree of endemicity. Rates in a period of 7-8 months in high, medium, and low endemic areas were 0.231, 0.083, and 0.022, respectively. The rate of nodule appearance in persons nodulectomized, or rate of nodules appearing in subjects who were surgically rendered negative during the first survey, was 45.3%, 36.4%, and 4.5% in those areas. The highest rate of nodule appearance was observed in patients with microfilariae in both the skin and nodules, followed by patients with either microfilariae or nodules. In highly endemic areas, new nodules appeared in 13.2% of persons without microfilariae or nodules, while in low endemic areas even patients with nodules earlier were less likely to have developed new ones. The performance of the nodulectomy teams (brigadas) was evaluated by confirming the worms in nodules. By gross examination alone, about 6% of nodules removed by brigadas did not contain worms. Nodules containing worms were examined for microfilariae to clarify their role as a source of microfilariae. Microfilariae were not detected in some of these nodules, especially in small ones. In contrast, microfilariae emerged from 80% of those larger than 10 mm. Number of worms per nodule, their sex, and the fecundity of female worms were examined by a collagenase technique. The average number of worms per nodule was 0.6 males and 1.2 females. The highest burden in any one nodule was seven worms, four females and three males. Most nodules had one female and either one male worm or none. Intrauterine microfilariae were found in most females from nodules shared with a male, while solitary females were not gravid. Solitary females were likely to be found in small nodules and large nodules were more likely to include both sexes.

Since 1933 a nationwide nodulectomy campaign has been conducted in Guatemala as a control measure, but not as radical treatment, of onchocerciasis;¹ all known endemic areas have been visited once or twice a year by paramedical nodulectomy teams or "brigadas." Although there is evidence of a reduced prevalence of onchocercal blindness,² overall morbidity appears to have remained unaffected.³ Romero and Wise questioned the effectiveness of mass nodulectomy as a means

of disease management, and stressed the need for a critical evaluation.⁴ Fuglsang and Anderson found that removal of head nodules was partially effective in reducing the microfilarial concentration in the cornea, but not in the skin, and was of some benefit to lesions of the anterior segment of the eyes.⁵ They noted, however, that removal of head nodules is far from sufficient as a curative measure in ocular onchocerciasis. Recently, Kale also reported that nodulectomy has no influence in microfilarial density.⁶ The general assumption that nodules, rather than worms free in the skin, are a significant source of microfilariae has not been examined thoroughly in Guatemala.

In this investigation, we examined two major unanswered questions relevant to the value of nodulectomy as a control measure: the role of the

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nodule as a source of microfilariae, and appearance of new nodules in a previously treated population.

MATERIALS AND METHODS

Survey

Since 1976 a research and control program against onchocerciasis has been carried out in and around San Vicente Pacaya (SVP), Guatemala. In these areas, we were able to observe the rate of nodule appearance in a given period and to examine parasite contents of nodules during an epidemiological survey conducted between 1976 and 1980. In the initial survey conducted in 1976, almost half of the total population in SVP were interviewed on their history of nodulectomy, to ascertain the extent of work done by brigadas before our survey. Residents were then palpated for nodules, skin snipped, given an anterior chamber examination by slit lamp, and all detected nodules were removed. The morbidity of disease, population and topography of SVP have been published in our previous paper.⁷ Twelve villages and plantations surveyed between June and September in 1976 were selected as the study area for appearance of new nodules. The total population of areas selected was about 2,000. A follow-up survey was conducted between January and April in 1977, 7–8 months after the first survey. At the second survey the inhabitants were re-examined for nodules and nodulectomy was done where needed, but skin snips were not taken. In our study, all nodules diagnosed and removed by brigadas were regarded as of onchocercal origin. To determine the rate of appearance of new nodules, only persons examined in both surveys were used. The rate of new nodules appearing in the initially negative subjects was expressed as an incidence rate, based on a period of 7–8 months; this was defined as the number of subjects with new nodules in the second survey divided by the total number of initially negative subjects in the first survey. The rate of new nodules appearing in persons surgically rendered negative may be regarded as “the rate of nodule appearance in the nodulectomized.” This was calculated as the number of subjects with new nodules in the second survey divided by the total number of subjects surgically rendered negative among nodule positives in the first survey. The study areas arbitrarily classified into three zones according to skin microfilaria positive rate

(MF) at the time of the first survey in 1976. MF, nodule positive rate (NPR) and microfilaria positive rate in anterior chamber of the eyes (MFAC) in each of three zones were as follows: High endemic areas, MF, 62.3–80.3%, NPR, 3.46–73.3%, MFAC, 6.7–27.9%; Medium endemic areas, MF, 35.6–38.7%, NPR, 17.8–34.4%, 3.8–10.2%; Low endemic areas, MF, 7.6–18.2%, NPR, 9.7–15.1%, MFAC, 0–1.4%.

Examination of nodules

A total of 888 nodules were examined; 591 nodules collected in 1977, 179 of which were new nodules obtained at follow-up survey described previously, and 297 nodules collected in 1979–1980. The nodules were measured using callipers after removal of their loose connective tissue, and the size of each was expressed as the arithmetic mean of their three dimensions. To evaluate the diagnostic certainty in palpation of nodule by brigadas, nodules collected in 1977 were cut into several pieces using surgical blade and examined for worms under dissecting microscope. At least 555 (94%) of 591 nodules examined were confirmed to contain the worm, without the use of histological techniques. The nodules containing worms were minced and soaked in saline for 30 min to allow microfilariae to emerge. In 1979 and 1980, whole nodules were kept on ice for 2–3 days, followed by collagenase digestion⁸ to determine the number of worm per nodule, their sex, and the fecundity of female worms. Of 297 nodules examined, 276 (93%) contained at least one worm. Sixteen nodules contained calcified or fragmented worms and were excluded from calculation. Recovered male worms were intact, but perhaps other broken ones escaped detection. Most female worms recovered were severed in at least several points.

RESULTS

Survey data

Pre-1976 nodulectomy histories (Table 1) indicated that the proportion of those receiving at least one nodulectomy increased with age, to a maximum of almost 75% in the over 20 age group. The highest proportion of nodulectomized individuals was observed in the area of maximum endemicity.

Of the 1,631 subjects screened in 1976, 874 (53.5%) were re-examined in 1977 (Table 2). The nodules were detected in 235 (26.9%) of 874 sub-

TABLE 1

Patient histories of onchocercal nodulectomy performed by brigadas prior to 1976 in San Vicente Pacaya, Guatemala, summarized by age and degree of endemicity

Degree of endemicity*	Proportion of persons surveyed reporting at least one nodulectomy			
	Age (yrs)			Total
	0-9	10-19	≥20	
High	29/69 (42.0)†	34/48 (70.8)	59/79 (74.7)	122/196 (62.2)
Medium	10/209 (4.8)	48/157 (30.6)	139/308 (45.1)	197/674 (29.2)
Low	0/129	6/86 (7.0)	39/166 (23.5)	45/381 (11.8)

* See Materials and Methods.

† No. reporting nodulectomy/no. questioned (% reporting).

jects in the first survey and 138 (15.8%) in the second survey. The average number of nodules per person was reduced from 1.77 in 1976 to 1.38 in 1977. Of 639 persons initially without nodules, 46 (7.2%) showed new nodules. Again, the highest incidence rate was observed in the inhabitants of highly endemic zones. Among 226 persons surgically rendered nodule-negative in 1976, the rate of appearance of new nodules was about fivefold (36.7%) that seen in the initially negative group.

When 1976 examinees were classified as positive or negative by skin snip and nodules (Table 3), the highest rate of nodule appearance was observed in the patients with both microfilariae in the skin and nodules, in high and medium endemic areas. In the low endemic areas, even patients with onchocercal signs were less likely to show new nodules. In the highly endemic areas, new nodules appeared among 13.2% of persons initially without microfilariae and nodules.

Examination of nodules

The sizes of nodules collected from villages surveyed between April and July in 1977 ranged from

3.3–18.0 mm with mean of 8.2 ± 2.7 mm ($n = 388$). Microfilariae were not detected in some nodules, even those with worms; detection rate of microfilariae increased with nodule size (Table 4). Microfilariae were not detected in nodules smaller than 3.9 mm. They emerged from one-fourth of those 4.0–5.9 mm and from 81% of those greater than 10 mm. The new nodules obtained at the follow-up study between January and April in 1977 varied from 3.5 mm–19.7 mm with mean of 7.4 ± 2.8 mm ($n = 136$) in size. Microfilariae emerged from 30% of those nodules smaller than 5.9 mm, from 59% of those between 6.0 and 9.9 mm and from 89% of those greater than 10 mm.

Of 484 total identifiable worms recovered from 260 onchocercomas, 66.7% were female and 33.3% were male. Measurable males averaged 2.5 ± 0.6 cm in length (1.4–4.5 cm) and females averaged 32.9 ± 11.2 cm (8.9–69.7 cm). When nodules were categorized by number and sex of enclosed worms (Table 5), it was apparent that the majority of nodules (81.2%) had only one female worm; the great majority likewise had either one or no male worms and higher numbers were progressively more unlikely. The average number of worms per

TABLE 2

Comparison of results of two onchocercal nodule surveys in San Vicente Pacaya, Guatemala, by presence or absence of nodules and by degree of endemicity

Degree of endemicity*	Nodules (1976) Nodules (1977)	No treatment			Nodulectomy		Total no. surveyed	Incidence rate†	Rate of new nodule appearance in nodulectomized persons‡
		Neg.	Neg.	Pos.	Pos.	Pos.			
		Neg.	Pos.	Pos.	Neg.	Pos.			
High		50§	15	3	47	39	154	0.231	0.453
Medium		278	25	5	75	43	426	0.083	0.364
Low		265	6	1	21	1	294	0.022	0.045
Total no. surveyed		593	46	9	143	83	874	0.072	0.367

* See Materials and Methods.

† Includes only nodule-positives on the second survey who were initially negative.

‡ Includes only the subjects with new nodules on the second survey who were surgically rendered negatives on the first survey.

§ Number of subjects examined in both surveys.

TABLE 3

Comparison of results of two onchocercal nodule surveys in San Vicente Pacaya, Guatemala, by presence or absence of microfilariae in skin and nodules, and by degree of endemicity

Degree of endemicity*	Microfilariae in skin and nodules in 1976		No. of cases	No. positive for nodules in 1977 (%)
Low	MF+(-)	N‡(-)	252	5 (2.0)
	Mf (-)	N (+)	13	1 (7.7)
	Mf (+)	N (-)	19	1 (5.3)
	Mf (+)	N (+)	9	0
Medium	Mf (-)	N (-)	242	12 (5.0)
	Mf (-)	N (+)	32	5 (15.6)
	Mf (+)	N (-)	61	13 (21.3)
	Mf (+)	N (+)	86	38 (44.2)
High	Mf (-)	N (-)	38	5 (13.2)
	Mf (-)	N (+)	11	4 (36.4)
	Mf (+)	N (-)	27	10 (37.0)
	Mf (+)	N (+)	75	35 (46.7)

* See materials and methods.

† Microfilariae in skin.

‡ Nodules.

nodule was 0.6 males and 1.2 females. The highest burden in any one nodule was seven worms, four females and three males. Almost half of 260 nodules contained at least one worm of either sex. With a single exception, all unisexual nodules contained a female worm. Only one unisexual nodule containing a male worm was found.

Examination of sex distribution with respect to nodule size showed that solitary females were likely to be found in small nodules and that larger nodules were more likely to include both sexes. Nineteen percent of all nodules smaller than 5.9 mm contained worms of both sexes, while 62.6% of those exceeding 8 mm represented both sexes. In a sample of 100 female worms, the male's pres-

TABLE 4

Relationship between nodule size and presence of microfilariae*

Size of nodule (mm)	No. nodules examined (frequency distribution: %)	No. nodules positive for microfilariae (%)
<3.9	4 (1.0)	0
4.0-5.9	79 (20.4)	20 (25.3)
6.0-7.9	125 (32.2)	75 (60.0)
8.0-9.9	90 (23.2)	66 (73.3)
10.0-11.9	44 (11.3)	35 (79.5)
12.0<	46 (11.9)	38 (82.6)
Total	388	234 (60.3)

* Of 412 nodules examined, 24 contained no worms and were excluded from calculation.

TABLE 5

Two-way frequency distribution of nodules with respect to the number and sex of enclosed *O. volvulus*

No. female worms/nodule	No. male worms/nodule						Total nodules
	0	1	2	3	4	5	
0	0	1	0	0	0	0	1
1	118	80	10	2	0	1	211
2	12	16	6	2	0	0	36
3	2	3	3	0	0	0	8
4	0	3	0	1	0	0	4
Total nodules	132	103	19	5	0	1	260

ence was associated with the appearance of intrauterine microfilariae. Intrauterine microfilariae were found in most (85.4%) of 41 females from nodules shared with males, whereas all of 59 solitary females were not gravid.

DISCUSSION

So far, the nodule rate has been used as the index of endemicity of onchocerciasis in Guatemala.⁹ In SVP, Guatemala, we have previously showed that about 16% of those infected were nodule-positive and were negative for cutaneous or ocular microfilariae. Therefore, nodule palpation is a valuable diagnostic procedure, especially among children in Guatemala. Detection and removal of nodules in Guatemala is performed by experienced paramedical personnel called brigadas, during their annual or semiannual visits. The brigadas' work before our first survey is presented in this study. The percentage of nodulectomized population in our study area was especially high (62%), in highly endemic areas. It appears that new nodules can be formed in a period of 6 months.

The rate of appearance of new nodules in Guatemala was first established by follow-up study. Both the incidence rate and rate of nodule appearance in the nodulectomized were correlated with the degree of endemicity. The degree of endemicity in SVP was reported to be altitude-dependent, and the highest microfilaria rate was found in villages situated between 600 and 900 m above sea level.⁷ In highly endemic areas, the incidence rate was 0.231, and 45.3% of those nodulectomized again presented nodules within 7-8 months. The highest rate of nodule appearance was observed in the patients with microfilariae in the skin and nodules, followed by patients with

either microfilariae or nodules, in high and medium endemic areas. In highly endemic areas, new nodules appeared among 13.2% of persons without microfilariae or nodules. In low endemic areas, even the patients with either microfilariae or nodules were less likely to show the new nodules. The higher rate of nodule appearance probably represents more frequent exposure to vector flies in highly endemic areas.

The rate of nodule appearance might be affected by the erroneous diagnosis by brigadas. To evaluate the performance of brigadas in the present study, the nodules removed by brigadas were examined for worms. Thirty-six (6.1%) of 591 nodules examined did not harbor *Onchocerca volvulus*. Even some of the nodules without apparent worms might have also been onchocercal, as no histological technique was applied. The frequency of misdiagnosis (6%) can be regarded as inevitable, because it is sometimes difficult to distinguish other tumors from onchocercomas by palpation, especially when they are small.

To date, little is known about the stimuli which provoke nodule formation. They may develop in response to dead worms,¹⁰ or they may represent a unique response to mature adults.¹¹ According to Schulz-Key, *O. flexuosa* nodules are induced by the immature stage of the parasite.¹² Recently Collins et al.¹³ observed that the nodules form only around female worms. Nodules detected in our re-survey presumably represented the growth of nodules which had been too small to be palpated, the encapsulation of worms free in tissue at the time of the first survey, or perhaps the encapsulation of worms transmitted by flies since the first nodulectomy.

Our nodules differ from those studied in Africa¹⁴ in a number of respects. Guatemalan nodules were much smaller, contained about half as many worms, and these worms were rarely calcified (less than 2%). About half the African nodules measured less than 10 mm and one third were less than 8 mm. Our corresponding figures were 76.8% and 53.6%. African nodules were more likely to contain both sexes (82.7%) than Guatemalan (48.8%). These differences may reflect the active nodulectomy campaign in Guatemala. Even though examined when fresh, a high proportion of Guatemalan nodules (40%) were without detectable microfilariae. Small nodules were less likely to contain microfilariae than large ones. About half our nodules contained only solitary, nongravid females but a high proportion of fe-

males sharing nodules with males (85.4%) were gravid. We also noted that the proportion of nodules containing both sexes increases with nodule size. Similar observations have been reported on the Australian cattle onchocercid, *O. gibsoni*.¹⁵ Contrary to our experience, gravid females in nodules without males have been observed in human^{13,14} and animal¹⁵ onchocerciasis. Perhaps male worms move among nodules,¹² worms are overlooked,¹⁵ or mating occurs before or early during the formation of nodules.¹³

It is generally believed that microfilariae leave the nodule,¹⁶ and spread into the dermis and eyes. The rationale behind mass nodulectomy campaigns has been that repeated nodulectomy would reduce the skin density of microfilariae. In one small village with a population of about 160 and microfilaria rate of 85%, we removed all detectable nodules from 10 patients and then examined quantitatively the change of concentration of microfilariae in the skin every 2 months for one year. Each of the subjects had at least one nodule containing both sexes of worm. Six months after the nodulectomy, six patients developed new nodules which were again removed. The change of microfilarial density seemed quite variable and in none of the patients was a significant tendency to decrease shown within the period of observation (unpublished data). Fuglsang and Anderson,⁵ and Kale,⁶ report a similar experience after removal of African nodules. Effectiveness of nodulectomy is perhaps masked by several factors, such as the activity of deep, unpalpable nodules,¹⁷ adult worms lying free in the subcutis,¹⁸ a long microfilarial life span,¹⁹ or uneven distribution of dermal microfilariae, even in adjacent locations.²⁰ We failed to demonstrate a direct efficacy of nodulectomy on microfilarial density in the skin, but large nodules can be stressed as the significant source of microfilariae, which are the pathogenic stage in onchocerciasis.

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