EVALUATION OF METHODS FOR DIAGNOSIS OF MICROFILARAEMIA ON KINMEN (QUEMOY) ISLANDS

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INTRODUCTION

In the endemic area of bancroftian filariasis of Kinmen (Quemoy) Islands the filarial carriers were routinely detected by making a thick smear on a glass slide (2.5 × 7.5 cm) of about 20 c.mm night blood from the finger and then identifying stained microfilariae under microscope (Brady and Lawton, 1944). However, a number of cases with low microfilarial density might be missed owing to the small blood volume used (Desowitz *et al.*, 1970). Therefore, under such circumstances, concentration methods including millipore membrane filtration (Bell, 1967; Chularerk and Desowitz, 1970) and Knott's techniques have been employed. The merits of these three methods are discussed herein.

MATERIALS AND METHODS

This investigation was performed intermittently from January, 1972 to July, 1973.

Two or 5 ml of blood was drawn from cubital vein from each of 420 Kinmen Chinese with venoject tubes containing EDTA powder as anticoagulant (Jintan Termo Co., Ltd., Tokyo, Japan). The blood samples were kept in ice box and sent within 48 hours by air to the laboratory in Taipei, and used to perform millipore membrane filtration and Knott's methods for concentration examination for microfilariae.

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For millipore membrane filtration technique, 0.5 or 1 ml of the 2 ml or 5 ml of EDTA-treated venous blood with viable microfilariae was drawn up into a 10-ml syringe. A 10% solution of Teepol (Shell Chemicals, Tokyo, Japan) in normal saline was then sucked into the syringe up to 10 ml and mixed with the blood by gentle rotation for about one minute until the blood was completely haemolyzed. The needle was removed and replaced by a 25-mm circular filter holder containing a 25-mm membrane filter of 5 μ porosity (Millipore Corporation, Massachusetts, U.S.A.). By gently pushing the syringe piston, all the haemolyzed blood was forced through the filter. The membrane was washed by passing 5 ml of normal saline through it, followed by 5 ml of hot water (around 90°C) to fix any presenting microfilariae. The membrane was then removed from the holder and placed in 3% Giemsa solution for 1 hour, washed in tap water, and allowed to dry. Then it was placed on a glass slide, mounted in Gurr's green neutral mounting medium (George T. Gurr Ltd., London, England). The well prepared membrane was examined microscopically for presence of microfilariae and their number.

For Knott's method, another 0.5 or 1 ml of the remaining EDTA-treated venous blood was transferred with a 1-ml graduated pipette into a 15-ml centrifuge tube containing 8 ml of 2% formalin in normal saline. The pipette was washed twice by drawing up 1 ml each time and transferring the formalin solution into the centrifuge tube. Thus in the

centrifuge tube there were 1 ml of blood and 10 ml of 2% formalin solution the dilution was 1 in 10. The solution was then mixed by gentle rotation, let to stand for 10 minutes, and centrifuged (1500 r.p.m.) for 3 minutes. After carefully discarding the supernatant fluid, the sediment was stained with 3% Giemsa in bulk for 1 hour, then completely taken out with capillary pipette to make a smear (2 × 3 cm in area) on a glass slide. The centrifuge tube and capillary pipette were once more washed with 10 ml normal saline. The washed solution was centrifuged and the sediment used to make up another smear. The smears were examined microscopically for presence and number of microfilariae.

While the venipuncture was being performed, from each of the participants 20 or 100 (20 × 5) c. mm of finger-pricked blood was obtained with haemoglobin pipette for thick smear examinations. The blood smear was dried in the open air, dehaemoglobinized and stained at the same time with 3% Giemsa solution for 1 hour, and then examined, microscopically for presence and number of microfilariae.

RESULTS

Sixty-two microfilaremia cases were found by combination of millipore membrane filtration, Knott's and thick smear methods (Table 1). Among the cases (No. 1 to No. 42) the microfilarial carriers were basically diagnosed by millipore membrane filtration technique with 0.5 ml venous blood taken at 2200-2300 hours (average of 169.6 microfilariae per 0.5 ml blood); but by thick smear method with 20 c.mm finger-tip blood taken at 2200-2300 hours (76.2% positive; average of 9.5 microfilariae per 20 c.mm blood), the positive rate was comparatively low. Among the cases (No. 43 to No. 53) thick smear method (20 c.mm finger-tip blood at 2200-2300 hours), Knott's and millipore membrane

Table 1

Microfilarial examination on the 62 positive cases showing methods and volume of blood used, hour of collection, and number of microfilariae found.

Case No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
20 c.mm "F"	2200-2300	47	39	30	30	27	24	22	19	17	16	14	12	12	12	10
0.5 ml "M"	2200-2300	410	160	361	82	110	458	380	332	54	1664	328	362	221	52	172
Case No.		16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
20 c.mm "F"	2200-2300	9	7	7	7	6	6	5	3	3	3	3	2	2	2	2
0.5 ml "M"	2200-2300	140	159	74	26	106	88	336	90	34	22	13	131	113	69	55
Case No.		31	32	33	34	35	36	37	38	39	40	41	42	Av.	% pos.	
20 c.mm "F"	2200-2300	2	1	0	0	0	0	0	0	0	0	0	0	9.5	76.2	
0.5 ml "M"	2200-2300	8	16	171	143	118	24	12	12	9	4	2	1	169.6	100.0	
Case No.		43	44	45	46	47	48	49	50	51	52	53	Av.		% pos.	
20 c.mm "F"	2200-2300	130	17	4	4	3	3	3	2	1	70	0	21.5		90.9	
1 ml "K"	2200-2300	1114	197	198	120	116	107	90	11	130	2155	6	388.5		100.0	
1 ml "M"	2200-2300	1460	70	414	46	347	105	22	10	63	1120	5	333.0		100.0	
Case No.		54	55	56	57	58	59	60	61	62	Average			% positive		
20 c.mm "F"	0900-1100	0	0	0	0	0	0	0	0	0	0			0		
1 ml "M"	0900-1100	0	1	3	0	0	0	1	0	0	0.6			33.3		
20 c.mm "F"	2200-2300	54	14	8	6	4	4	2	2	1	10.6			100.0		

"F" = finger-tip blood for thick smear examination; "K" = venous blood for Knott's method; "M" = venous blood for millipore membrane filtration technique.

Table 2

Microfilarial examination on 18 positive cases after hetrazan treatment by consecutive five blood smears, Knott's and millipore methods at 2200 hours.

Case No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Av.	% pos.
1st 20 c.mm	3	2	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0.4	27.8
2nd 20 c.mm	1	3	0	0	0	2	1	1	1	1	0	0	0	0	0	0	0	0	0.6	38.9
3rd 20 c.mm	2	2	0	0	0	0	0	2	0	0	1	1	0	0	0	0	0	0	0.4	27.8
4th 20 c.mm	0	1	1	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0.3	27.8
5th 20 c.mm	1	2	6	0	0	5	2	0	0	0	0	0	0	0	0	0	0	0	0.9	27.8
Total 100 c.mm	7	10	8	2	1	7	4	3	2	1	1	1	0	0	0	0	0	0	2.6	66.7
1 ml (millipore)	10	112	30	55	96	7	27	4	4	30	54	5	7	6	2	2	1	0	25.1	94.4
1 ml (Knott's)	40	41	4	24	10	13	23	9	5	18	6	6	3	3	4	1	0	1	11.7	94.4

filtration methods (each 1 ml venous blood at 2200-2300 hours) were used for diagnosis. Knott's method and millipore membrane filtration technique gave no remarkable different results (both with 100% positive rate and microfilarial density, 388.5 and 333.0 per 1 ml blood respectively). But the thick smear method, because of small blood volume examined, gave a lower positive rate (90.9% positive; average of 21.5 microfilariae per 20 c.mm blood). Examination of daytime blood (0900-1100 hours) of 9 positive cases (No. 54-62) those diagnosed by thick smear method (20 c.mm finger-tip blood) at night-time revealed that only 3 cases were detected by millipore membrane filtration technique (33.3% positive; average of 0.6 per 1 ml blood) and none by thick smear method.

Follow-up examination of carriers after hetrazan (diethylcarbamazine citrate) treatment revealed that routine thick smear method, even with consecutive 5 blood smears, was insufficient to detect the carriers with low microfilarial density (Table 2).

DISCUSSION

Diagnosis of microfilaraemia depends much on the volume of blood examined. The larger the volume used for examination, the higher is the accuracy of diagnosis (Desowitz *et al.*, 1970; Denham *et al.*, 1971; Dennis and Kean, 1971). That is why the millipore membrane filtration and Knott's techniques give a higher positive rate for microfilaraemia. However, these two methods consume more time, and can be performed accurately only in the laboratory. But in a mass survey, from the standpoint of paucity of equipment and simplicity, the thick smear method was the first choice. Moreover, the reluctance of people to submit to venipuncture, unless under compulsion, makes millipore membrane filtration and Knott's techniques well-nigh impracticable for mass survey in field work.

Because of nocturnal periodicity of bancroftian microfilariae in Kinmen area (Fan and Hsu, 1957), examination of day blood usually failed to reveal microfilaraemia cases by thick smear method, and occasionally by the millipore membrane filtration method. Nocturnal blood obtained by finger-prick for thick smears was found to be the most suitable for mass survey of microfilaraemia. In a previously hetrazan treated area, a bigger blood volume up to 60 c. mm or more can be obtained if greater accuracy demands (Edeson, 1959). Concentration methods including millipore membrane filtration and Knott's are recommended for follow-up examination of post-hetrazan treated cases.

SUMMARY

Methods including millipore membrane filtration, Knott's and thick smear have been used in filariasis endemic area of Kinmen Islands for diagnosis of microfilaraemia. The first two methods gave a higher positive rate because of larger volume of blood being examined. But the reluctance of people to accept venipuncture and complexity of performance preclude these two methods from routine survey use. Although thick smear examination of finger-prick blood at night gave a lower positive rate, the simplicity and high acceptability made this method highly practicable in mass examination for microfilaraemia cases on Kinmen Islands. For detection of microfilarial carriers after hetrazan treatment, concentration methods are recommended.

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