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Full Length Research Paper

Detection of microfilariae with counting chamber technique in some Nigerian rural communities

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The conventional stained thick smear technique (TS) has innate possibility of underestimation of microfilariae (mf) in microfilaraemic subjects. The usability of counting chamber (CC) technique in the detection of mf was determined in three Nigerian rural communities where *Loa loa* and *Mansonella perstans a*re endemic. Blood samples were collected by finger-pricking method from 612 subjects (334 males, 278 females), between February 1996 and July 1998. Each blood sample was examined for mf using TS or/and CC technique(s). CC technique had statistically higher sensitivity (79.3%) than TS technique (39.1%). For both techniques, the microfilarial range was 1 to 20 mf/50 µl of blood. There was no statistically significant differences between the microfilarial geometric mean intensities with both techniques.

Key words: *Loa loa*, *Mansonella perstans*, microfilaraemia detection, counting chamber technique, Nigeria

INTRODUCTION

The simultaneous occurrence of human *Loa loa* and *Mansonella perstans* infections is an established phenomenon, which has been documented in different parts of Nigeria (Udonsi, 1988; Ufomadu et al., 1991; Anosike, 1994; Agbolade and Akinboye, 2001). Safe chemotherapeutic control of these filarial infections requires a proper estimation of the microfilarial intensities in infected patients. This is to prevent severe reactions which are common in patients heavily infected with microfilariae (mf) (Cheesbrough, 1987; Ottesen, 1990). Some previous studies have shown higher microfilarial sensitivities with counting chamber (CC) technique compared to the conventional stained thick smear (TS) technique (Denham et al., 1971; McMahon et al., 1979). However, no previous study involving the use of CC technique in Nigeria is known to the authors.

The present study aimed to determine the usability of CC technique in the detection of mf in some Nigerian rural communities where *L. loa* and *M. perstans* are endemic.

MATERIALS AND METHODS

Study area and questionnaire administration

The study area consisted of Abata, Awori-Jeje and Mamu villages in Ijebu North Local Government Area of Ogun State, Nigeria, and has been previously described (Agbolade and Akinboye, 2000, 2001; Agbolade, 2002). The consents of subjects who were examined in this study were duly obtained. For every participating subject, a questionnaire was used to obtain information such as sex, age, duration of residence, and history of worm crossing the eye (eyeworm).

Collection and examination of blood samples

Six hundred and twelve subjects were included in the study which was carried out from February 1996 to July 1998. The age range of the subjects was two to 65 year. The subjects were divided into

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Table 1. Microfilaraemia sensitivities of counting chamber (CC) and stained thick smear (TS) techniques.

Study group	No. examined	No. microfilaraemic*	Technique	No. positive (%)	No. negative (%)
A	132	61	CC	53 (86.9)	8 (13.1)
			TS	21 (34.4)	40 (65.6)
B	126	31	CC	20 (64.5)	11 (35.5)
			TS	15 (48.4)	16 (51.6)
Total	258	92	CC	73 (79.3)	19 (20.7)
			TS	36 (39.1)	56 (60.9)

* Total microfilaraemic cases with both techniques

Table 2. Prevalence and intensity of microfilariae in relation to counting chamber (CC) and stained thick smear (TS) techniques.

Study group	No. examined	Technique	Prevalence (%)	Intensity (mf/50µl)	
				Range	Geometric mean
A	132	CC	40.2	1 - 4	1.4
		TS	15.9	1 - 20	2.8
B	126	CC	15.9	1 - 14	3.1
		TS	11.9	1 - 2	1.3
C	354	CC	17.2	1 - 20	3.6

three study groups (A, B, and C) based on mf detection technique(s) used and periods of blood sample collection. Study group A consisted of 132 subjects (62 males, 70 females) whose blood samples were examined simultaneously for mf using CC and TS techniques. Study group B consisted of 126 subjects (60 males, 66 females) who though were examined for mf using both techniques, the blood sample collection for TS technique was done 1½ to 2 years after that for CC technique. Study group C consisted of 354 subjects (212 males, 142 females) who were examined using CC technique only.

Blood samples were collected between 11.00 and 14.00 h by finger-pricking method. For each subject in study groups A and B, 50 µl of blood was taken and used to make a thick smear. The thick smears were air-dried overnight, dehaemoglobinized, stained with buffered Giemsa stain (pH 7.2) and examined for mf as previously described by WHO (1987). For CC technique, 50 µl of blood was taken from each subject and discharged into a vial which contained 1 ml of 3% acetic acid. Each haemolysed sample was examined for mf using a counting chamber. For each technique, microfilarial intensity in each microfilaraemic subject was evaluated as previously described (Agbolade and Akinboye, 2001). Identification of filarial species was attempted with both techniques based on observable morphological characters.

RESULTS

The microfilaraemia sensitivities of CC and TS techniques in the study population are shown in Table 1. In study group A and the total (A + B) study population, CC had statistically higher sensitivity than TS ($P < 0.001$). But in study group B, there was no significant difference between the sensitivities of CC and TS techniques ($P > 0.05$). There was no correlation between positive and negative results with age.

The prevalences and intensities of mf as independently observed in the three study groups are shown in Table 2. In study group A, CC technique revealed a statistically higher prevalence than TS technique ($P < 0.01$), while the two techniques showed statistically similar prevalences ($P > 0.05$) in study group B. The total mf prevalence (28.3%) recorded with CC technique in study groups A and B was statistically similar ($P > 0.05$) with that recorded in study group C (17.2%). There were no statistically significant differences ($P > 0.05$) between the microfilarial geometric mean intensities observed with both techniques.

In study group A, 13 microfilaraemia cases were simultaneously detected with both diagnostic techniques. Out of these, one (7.7%) had equal intensities with both techniques. 11 (84.6%) had relatively higher intensities with TS technique, while two (15.4%) had relatively higher intensities with CC technique.

The distribution of microfilaraemic subjects in study group C based on microfilarial counts is summarized in

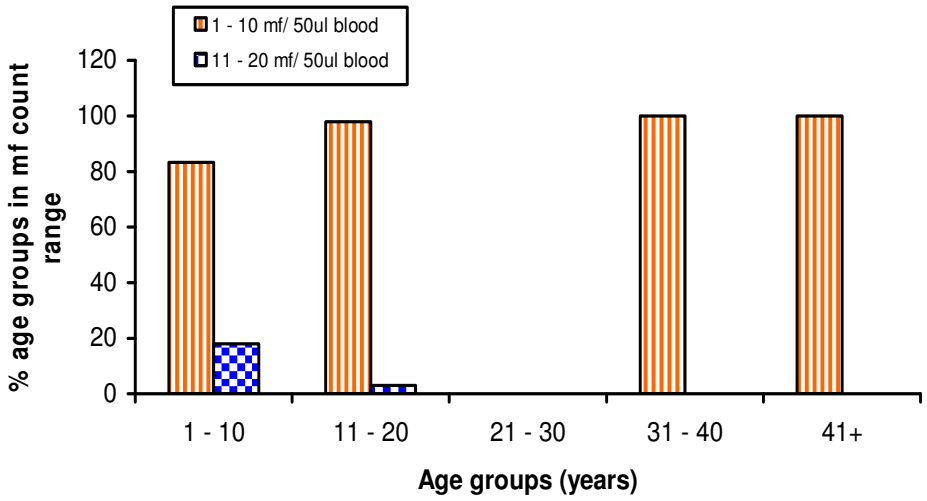

Figure 1. Distribution of microfilaraemic subjects in study group C according to microfilarial counts.

Figure 1. In all microfilaraemic age groups, at least 83.3% had microfilarial count range of 1 to 10 mf/50 µl of blood.

With TS technique, *L. loa* and *M. perstans* were indentified, but parasitological species identification was difficult with CC technique. Twelve (40%) of the 30 *L. loa* positive cases in study groups A and B had history of eyeworm. Seventeen (27.9%) of the 61 microfilaraemic subjects in study group C had history of eyeworm. The frequency of occurrence of eyeworm among *L. loa* positive subjects (40%) in study groups A and B was statistically similar to that among microfilaraemic subjects in study group C ($P > 0.05$).

DISCUSSION

This study has shown a higher microfilaraemic positivity sensitivity of CC technique than TS technique which is in conformity with previous findings (Denham et al., 1971; McMahon et al., 1979). However, the similar microfilarial geometric mean intensities with the two techniques might have been due to low microfilarial intensities of *L. loa* and *M. perstans* in the study area which have been reported earlier (Agbolade and Akinboye, 2001). The low microfilarial intensities might have contributed to the presence of relatively few higher intensities with CC compared with TS in study group A. Similarly, the low microfilarial intensities in the study area might have contributed to false negative cases with CC in this study.

The similar microfilaraemia positivity sensitivities of CC and TS techniques in study group B might have been caused, at least partly, by emergence of new microfilaraemia cases in the study group.

The problem of species identification with CC technique encountered in this study has been previously documented by some workers (Denham et al., 1971; WHO, 1987). Nevertheless, history of eyeworm has been closely associated with *L. loa* infection in endemic areas (Nutman et al., 1986; Ottesen, 1990; Takougang et al., 2002). In the present study, only 40% of the *L. loa* positive subjects had history of eyeworm. This suggests that no fewer than 27.9% of the microfilaraemic subjects in study group C of the present study, who had history of eyeworm, had *L. loa* infection. In this regard, a combination of CC technique and history of eyeworm provides some clue towards species identification and facilitates the use of CC which is known to be easy, cheap and time-saving (WHO, 1987). However, the associated problems of false negatives and lower microfilarial intensities recorded in this study suggest the need for further studies to establish its wide-scale usability in Nigeria.

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