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Case Report

Role of buffy coat in detection of microfilaria in absence of eosinophilia: a case report

Sailuja Maharjan ^{a,*}, Sumnima Mainali ^b, Akriti Shrestha ^c, Sabi Rana ^c, Srinivas Chakravarthy ^d

^a Department of Pathology, Kulhudhuffushi Regional Hospital, Kulhudhuffushi, Maldives

^b Department of Gynecology and Obstetrics, Kulhudhuffushi Regional Hospital, Kulhudhuffushi, Maldives

^c Department of ENT, Kulhudhuffushi Regional Hospital, Kulhudhuffushi, Maldives

^d Department of Laboratory Medicine, Kauvery Hospital, Chennai, India

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ABSTRACT

Filariasis is a very important public health problem endemic in tropical and subtropical countries. It is a debilitating disease with significant socioeconomic burden. In 2016, lymphatic filariasis was successfully eliminated from the Maldives, which was certified by WHO. We report a case of filariasis detected by a rapid antigen test while undergoing a screening program conducted by Health Protection Agency, Maldives in December 2023. The patient was asymptomatic and showed no peripheral blood eosinophilia. Conventional blood smears were negative. Nevertheless, buffy coat smear served as a promising test in detection of parasite, thus confirming the re-emergence of the disease in the country. Therefore, our study highlight the significance of buffy coat smears in detection of hemoparasites. We also focus on the importance of surveillance system in order to sustain the disease elimination goal that was already achieved.

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* Corresponding author.

E-mail address: sailuzaa73@gmail.com

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Papel de la capa leucocitaria en la detección de microfilarias en ausencia de eosinofilia: reporte de un caso

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RESUMEN

La filariasis es un problema de salud pública muy importante, endémico en países tropicales y subtropicales. Es una enfermedad debilitante con una importante carga socioeconómica. En 2016, se eliminó con éxito la filariasis linfática en Maldivas, lo cual fue certificado por la OMS. Informamos un caso de filariasis detectada mediante una prueba rápida de antígenos mientras se sometía a un programa de detección realizado por la Agencia de Protección de la Salud de Maldivas en diciembre de 2023. El paciente estaba asintomático y no mostraba eosinofilia en sangre periférica. Los frotis de sangre convencionales fueron negativos. Sin embargo, el frotis leucocitario sirvió como prueba prometedora en la detección del parásito, confirmando así la reaparición de la enfermedad en el país. Por lo tanto, nuestro estudio resalta la importancia de los frotis de capa leucocitaria en la detección de hemoparásitos. También nos centramos en la importancia del sistema de vigilancia para mantener el objetivo de eliminación de la enfermedad que ya se logró.

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1. INTRODUCTION

Filariasis constitutes a major public health problem and affects approximately 51.4 million population worldwide [1, 2]. Filariasis is a vector borne infectious disease seen predominantly in tropical and subtropical countries [3-5]. In 2016, lymphatic filariasis was successfully eliminated from the Maldives, which was certified by WHO [6, 7]. Herein, we report a case of filariasis detected incidentally by rapid antigen test during a screening program conducted by Health Protection Agency (HPA), Maldives in December 2023. The patient was asymptomatic and showed no peripheral blood eosinophilia. The thin and thick smears failed to demonstrate the parasite but was successfully detected in buffy coat smears, which helped in a definite diagnosis of the disease. Though the sensitivity of the rapid antigen test is high, it cannot distinguish between past and active infection [8]. The microscopic detection of the parasite remains the gold standard test, particularly in confirming the resurgence in filaria eradicated countries. Therefore, we highlight the significance of buffy coat smears in detection of hemoparasites.

2. CASE REPORT

A 31-year-old male patient tested positive while undergoing health campaign conducted by the Health Protection Agency

among the migrant workers in Kulhudhuffushi city, Haa Dhaalu atoll, the northern province of Maldives on December 2023. The examination was carried out in the department of pathology at Kulhudhuffushi Regional Hospital. Bioline filariasis test strip (Abbott diagnostics, Maine, USA) was used for the screening program. Retrospectively, the patient was enquired for history of fever, lymphadenopathy or any relevant past history but was negative. His general and systemic examination were normal. Hematological examination by SYSMEX XN-1000 series revealed Hb: 14.7gm/dL, TC: 7690/uL, DC: N53%, L40%, M4%, E3%, platelets: 329000/uL. Thick and thin blood smears were examined but did not demonstrate any parasites. Nevertheless, buffy coat analysis was also performed which detected microfilaria confirming the re-emergence of the infection in Maldives (Figure 1). The patient received a single course of combination of diethylcarbamazine, albendazole and ivermectin.

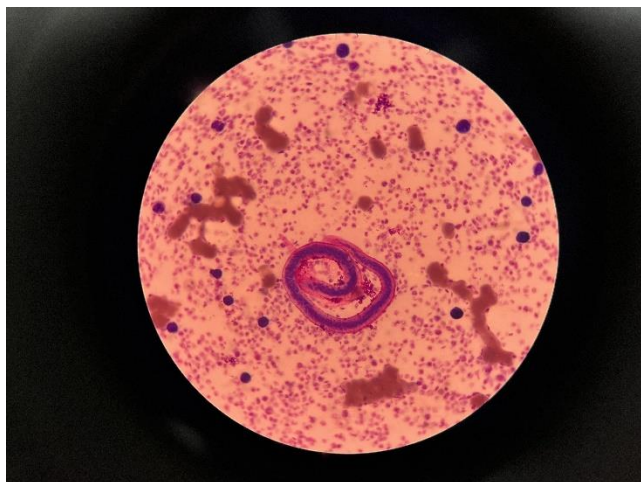


Figure 1: Buffy coat smear showing microfilaria (Leishman stain with 100X oil magnification).

3. DISCUSSION

Lymphatic filariasis is a major public health problem in many developing countries. It is a debilitating disease with significant socioeconomic burden in endemic areas. It is the second leading cause of permanent disability globally according to WHO [9]. Out of nine endemic south-east Asian countries, Maldives, Sri Lanka and Thailand were the only countries to have successfully eliminated filariasis as per WHO [6, 7]. However, the re-emergence of the disease in 2023 has triggered a major public health concern in Maldives following which the screening protocols have been meticulously reviewed. The diagnosis was confirmed by demonstration of the parasite microscopically in buffy coat smears, which further confirmed the re-emergence of the disease in the country. Our study also focuses the significance of surveillance system in order to sustain the elimination goal that was already achieved in endemic areas. Filariasis is a parasitic disease caused by three species of nematodes (*Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*) and transmitted by *Culex* mosquitoes. Around 95% cases of filariasis are caused by *W. bancrofti* which also accounts for the second most disabling disease after malaria among the diseases transmitted by mosquitoes [10-12].

The clinical spectrum of filariasis can vary from asymptomatic to acute or chronic phase [1, 2, 5, 10]. In clinically suspected cases, diagnosis can be made by circulating filarial antigen tests, fine needle aspiration cytology examination of peripheral blood smears or PCR techniques [4, 5]. Even with high parasitemia, the majority of the infected individuals remain asymptomatic throughout

their lives [3]. One of the clues to parasitemia in such cases is the presence of peripheral blood eosinophils (eosinophils >500/uL) [1]. It occurs as a hypersensitivity reaction to filarial antigen as the parasites migrate through the tissue. Therefore, careful screening of the peripheral blood smear should be done to detect the microfilaria in the background of eosinophilia. However, there are cases where microfilaria has been detected in the absence of eosinophilia as mentioned by Kesavan et al., Hakeem et al. and Ahuja et al [1, 5, 11]. The same finding was observed in our case. This could be attributed to factors like difference in host immune response to parasites, oxidative stress to the parasite during inflammation, co-infection with other diseases [3]. Likewise, Albhaisi et al. suggested that the absence of eosinophilia might be due to the altered immune status evoked by filariasis [12].

There are various methods for diagnosis of filariasis but the demonstration of microfilaria by microscopy is the gold standard test [1, 5]. In symptomatic patients with lymph node enlargement, FNAC is very helpful in identifying the microfilaria [4, 5]. Apart from lymph nodes, microfilaria has been demonstrated in aspirates from scrotal swelling, breast mass, ascitic fluid, synovial fluid and bone marrow [11-13]. Circulating filarial antigen test is a highly sensitive test, which is easy to perform with just a finger prick [4]. One of its advantage is that it avoids diurnal variation. However, due to its high cost, it may not be amenable to resource-limited endemic countries. Moreover, it fails to distinguish between past and active infection as the residual antigen maybe detected following elimination of the parasite after anti-filarial treatment [8]. Therefore, the definite diagnosis of active filariasis can be made by demonstration of the parasite, which was very crucial to confirm the re-emergence of disease in our case. The buffy coat smear examination appeared to be a very promising and powerful tool to demonstrate the parasite, as conventional blood smears were negative in our study. Buffy coat smear is prepared from peripheral blood, which is a very easy, sensitive and effective technique [14, 15]. It is based on the principle of centrifugal stratification of blood component and prepared by centrifugation of the EDTA blood sample at 1500g for 10 minutes [16]. This leads to deposition of platelets, WBC and parasites in the middle layer between supernatant plasma and sediment of RBCs. The utility of a buffy coat is well established in detection of hemoparasites like *Plasmodium*, *Leishmania*, *Trypanosoma* and microfilaria [15-17]. It is a very sensitive, cheap and effective method in resource limited areas to detect parasites to avoid false negative conventional blood smear results particularly with low parasitemia [14].

4. CONCLUSIONS

Our study illustrates that filariasis can exist without peripheral blood eosinophilia. It also highlights the importance of buffy coat films in detection of the parasites. We also urge to strengthen the monitoring programs to maintain the elimination target in endemic countries.

5. CONFLICT OF INTERESTS

The authors have no conflict of interest to declare. The authors declared that this study has received no financial support.

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