



DIAGNOSIS OF MICROFILARIAE IN HUMAN BY MODIFIED KNOTT TECHNIQUE IN IRAQ

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Abstract

The current study aimed to the detection of Microfilariae in humans for clinically unsuspected cases random samples and for different ages; Applied modified Knott technique for the first time in Iraq; used three types of stains for blood samples and compression the results. A total of 103 blood samples healthy and patient people, the current study revealed to 37 (35.92%) were infected with Filariasis. The current study recorded positive rates for four groups of ages: A. children, B. young, C. Adult and D. old. The statistic test revealed non-significant differences between ages of positive samples and for both genders with Filariasis. All of the stains were applied in current study appeared positive rates for infection (Methylene blue, Giemsa and Leshmen). This study recorded three genera of filariae according to the morphological characteristics (*Brugia*, *Mansonella* and *Wucheraria*).

Key words: Filariasis, Microfilaria, Knott technique, Dirofilaria, human.

Introduction

Filariasis is disease caused by parasitic worms that inhabit in the tissue and blood of humans and caused Lymphatic filariasis which called as Elephantiasis, (Kabaterine *et al.*, 2010).

Several genera of mosquitos may be indicative of transmitting of Filariasis including: *Mansonia*, *Ochlerotatus*, *Culex*, *Aedes* and *Anopheles*, (Burkot *et al.*, 2002). The females of mosquitoes are take up microfilariae from people when they take blood meals, this larvae needs about 12-15 days to develop into the mature stage in mosquitoes, (Scott, 2000). In all the world, Filariasis is caused by nematodes: *Brugia malayi*, *Brugia timori*, *Wuchereria bancrofti*, *Onchocerca volvulus*, *Mansonella perstans*, *Mansonella streptocerca*, *M. ozzardi*, *Loa loa*, *Dirofilaria conjunctivae*, *D. magalhaesi* and *D. immitis*. (Park, 2011; Hemali *et al.*, 2013). In Egypt, the relationships between *Wuchereria bancrofti* Microfilaria Counts and Maturation in *Culex pipiens* was studied by Farid *et al.*, 2003.

The aims of this study are: the detection of Microfilariae in humans for clinically unsuspected cases random samples and for different ages; Applied modified

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Knott technique for the first time in Iraq; used three types of stains for blood samples and comparison the results.

Materials and Methods

Collection of blood samples

A total of 22 blood samples were collected from different ages of healthy people randomly; 15 male and 7 female; from different regions in Baghdad city.

A total of 81 blood samples were collected from different ages of patient people randomly who undergoing treatments and surgeries at Ibn Al-Haytham Hospital for Ocular in Baghdad; 41 male and 40 female of different ages; from different provinces of Iraq.

Modified Knott techniques

All samples were put in gel tubes, with information include name, age and gender and then centrifuged; drop out the serum and put 1 ml of one of three stains: Methylene blue, Giemsa and Leishman stain. This study was conducted at Iraq Natural History Research Center and Museum, University of Baghdad, Iraq, during the period (January 2018 - July 2019).

Results

A total of 103 blood samples healthy and patient

people, the current study revealed to 37 (35.92%) were infected with Filariasis, table 1.

The current study recorded positive rates for four groups of ages: children (5-15 year), young (16-27 year), Adult (28-49 year) and old (50 year & above). That the higher rate 55.55% in children group. The statistic test revealed non-significant differences between ages of positive samples for Filariasis (Table 2).

The current study revealed out of 56 serum samples of male 19 were positive (33.92%) and out of 47 serum sample of female 18 were positive (38.29) for Filariasis. Furthermore, the statistic test revealed non-significant differences between male and female in groups of ages in Filariasis (Table 3).

The current study applied three types of stains Methylene blue, Giemsa and Lishmen for compression; all them appeared positive rates 38.15%, 35.71% and 23.07% respectively, table 4, fig. 1-10.

Table 1: Total rates of Filariasis infection in human by Knott technique.

No. of blood samples	No. of examined healthy people	No. of positive healthy people	No. of examined patient people	No. of positive patient people
103	22	0	81	37 (35.92%)

Table 2: Rates of Filariasis infection in human by Knott technique according to ages.

Age	No. of examined samples	No. of positive samples	%
A-Children	18	10	55.55
B-Young	19	6	31.57
C-Adult	29	9	31.03
D-Old	37	12	32.43
Total	103	37	35.92

Table 3: Rates of Filariasis infection in human by Knott technique according to gender.

No. of examined male sample	No. of positive male sample	%	No. of examined female sample	No. of positive female sample	%
56	19	33.92	47	18	38.29

* Non-significant differences between genders in groups.

Table 4: Rates of Filariasis infection in human by Knott technique according to stain used.

Stain name	No. of examined samples	No. of positive samples	%
Methylene blue	76	29	38.15
Giemsa	14	5	35.71
Lieshman	13	3	23.07
Total	103	37	35.92

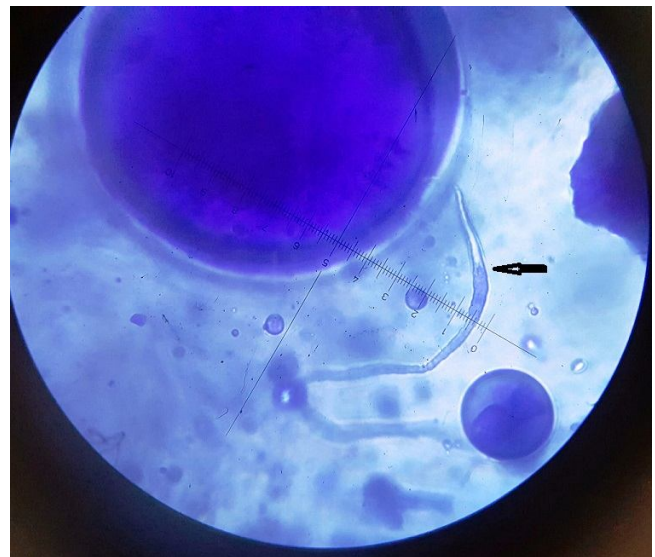


Fig. 1: Microfilaria in blood human stained with Methylene blue by Knott Technique, 10X.

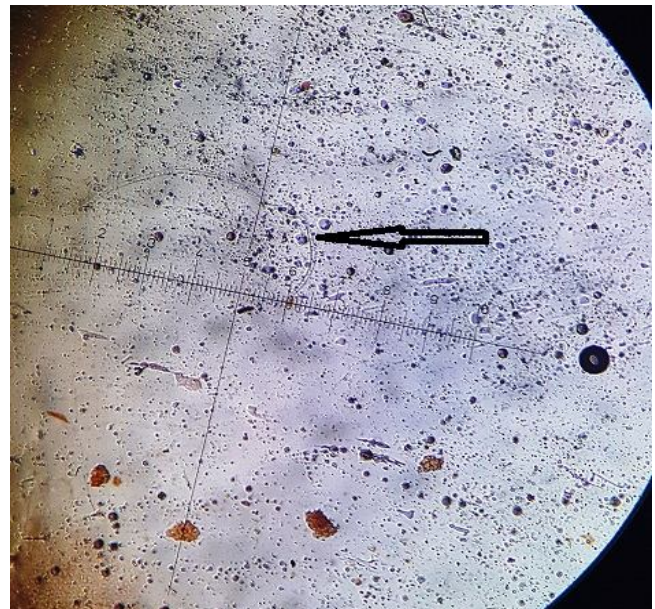


Fig. 2: Microfilaria not pigmented in blood human 6 years old stained with Methylene blue by Knott Technique, 10X.

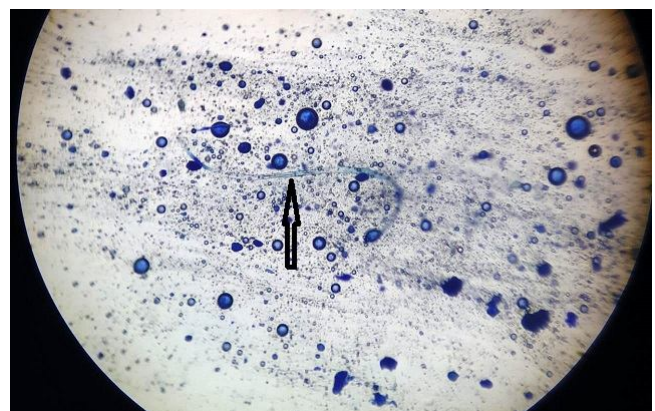


Fig. 3: Microfilaria in blood human stained with Leishman stain by Knott Technique, 10X.

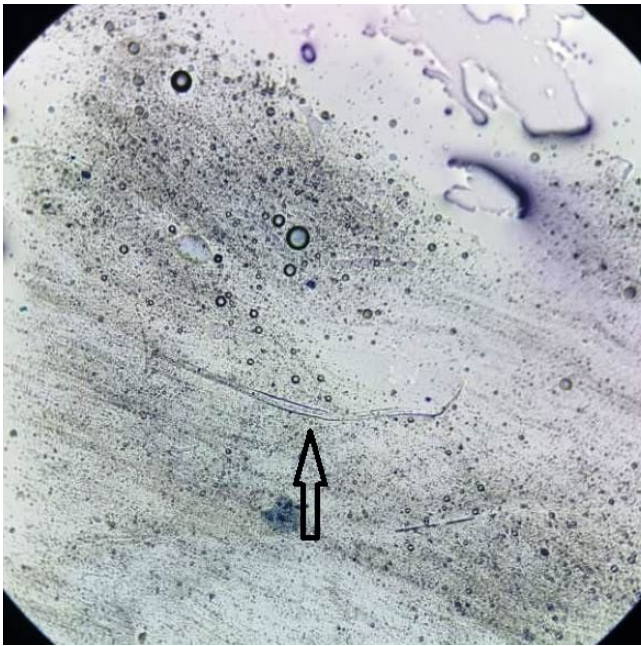


Fig. 4: Microfilaria not pigmented in blood human stained with Leshman stain by Knott Technique, 10X.



Fig. 5: Microfilaria in blood human stained with Giemsa stain by Knott Technique, 10X.

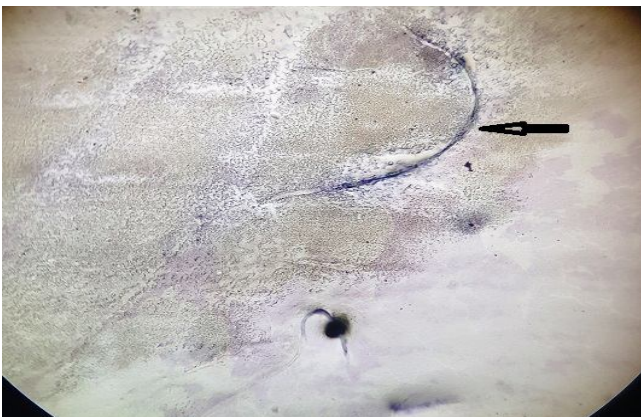


Fig. 6: Microfilaria in blood human stained with Giemsa stain by Knott Technique, 10X.

Discussion

Filariasis was considered as neglected tropical diseases that counted by WHO as 17 diseases; it is one of the important diseases for which control programs are developed, (WHO, 2013).

The concentration of the parasites in particular region is the best method for diagnosis as a modified Knott technique that a total of 103 blood sample healthy and patient people, the current study revealed to 37 (35.92%)

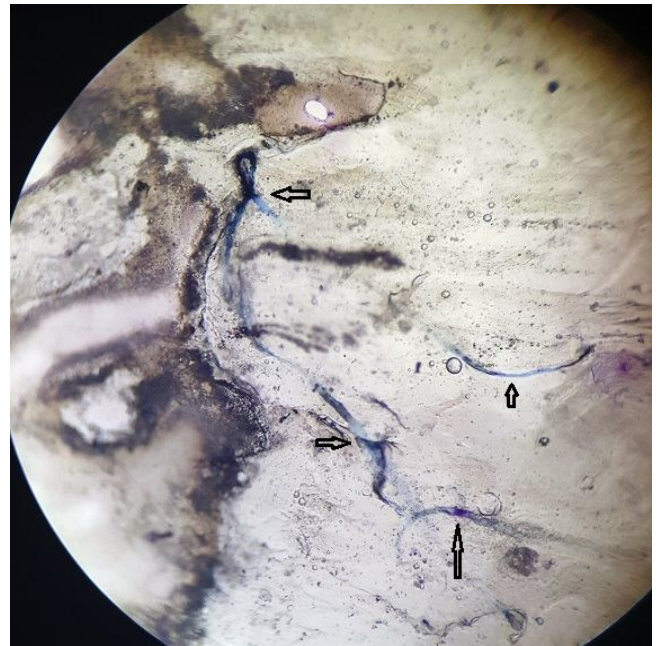


Fig. 7: Heavy infection of Filaraisis in child 13 years old stained with Methylene blue, 10X.

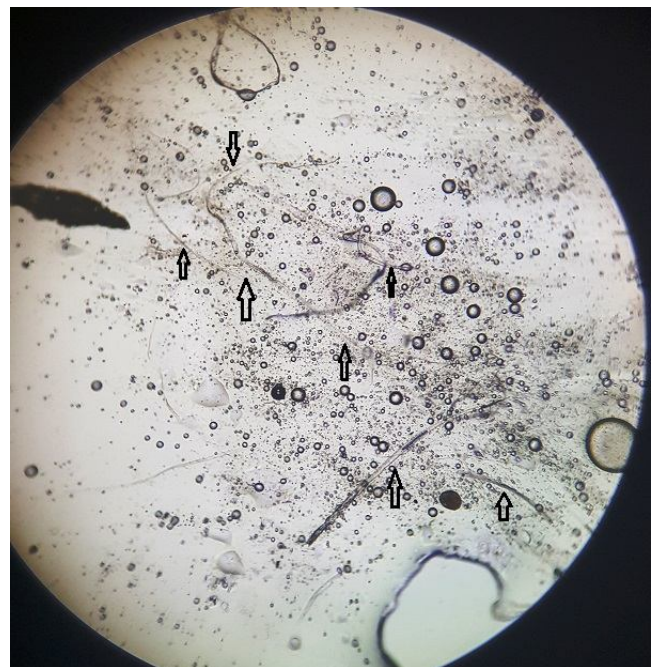


Fig. 8: Heavy infection of Filaraisis in old man 55 years old not stained with Methylene blue, 10X.

were infected with Filariasis. While, Santosh, (2017) revealed to only 10 cases from a total of 2926 by routine FNAC fine needle aspiration cytology smear and body fluids. That mean a modified Knott technique was the best for diagnosis of Microfilaria.

The study showed that all ages are exposed to infection with filariasis without a statistical difference. The first age group (children) showed that they are more susceptible to infection, that may because they more susceptible for biting by insects especially stable fly *Stomoxys calcitrans* which transmit of microfilariae (Hadi and Al-Amery, 2012). And also, non-significant differences between gender in groups that agreed with Alia *et al.*, (2013).

In Iraq, there are scanty of surveys and researches about microfilaria and their species; previously, Amall, (2010) recoded canine dirofilariasis and human dirofilariasis by Alia *et al.*, (2013). Equine filariasis was recorded by Alousi *et al.*, (1994) in Mosul and Hadi and Atiyah, (2014) in Baghdad. This spread hosts of microfilariae in Iraq may be due to the wide distribution of vectors as mosquitoes (Ubleis *et al.*, 2018) and stable fly (Hadi and Al-Amery, 2012).

All of the stains were applied in current study appeared positive rates for infection (Methylene blue, Giemsa and Lieshman), however, some cases appeared Microfilaria not pigmented for example: fig. 2 in blood of child 6 years old, figure 8 in old man 55 years old and

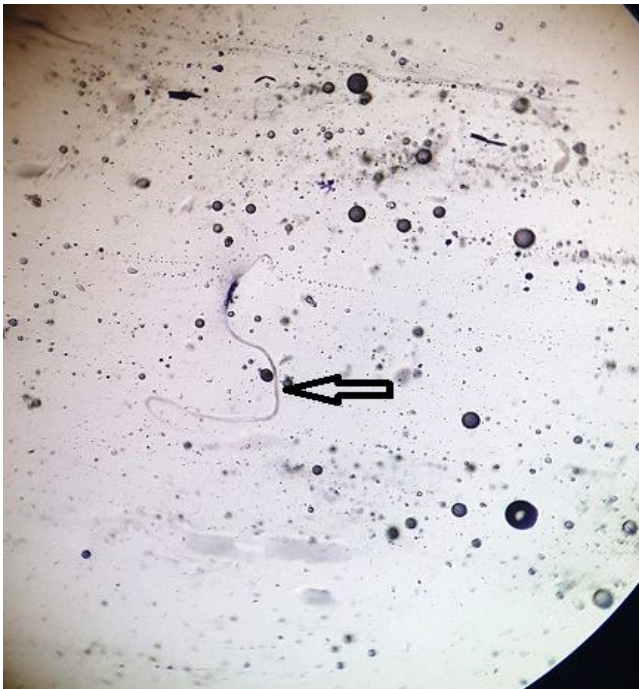


Fig. 9: Microfilaria in blood human of woman 50 years old not stained with Methylene blue by Knott Technique, 10X.

figure 9 in old woman. As you notice, neither age nor gender was the cause of unpainted specimens; that may be related to physiological parameter of blood specimens itself. While, Santosh, (2017) revealed to Giemsa stain is the definitive diagnosis method for filariasis in a peripheral smear.

The risk of infection with Filariasis that microfilaria has been detected at various unusual sites either associated with other diseases or independently in unsuspected cases, (Hemali *et al.*, 2013). As well as, many people remain reservoir of microfilariae at low levels after treatment, (Bryan and Southgate, 1976).

The current study revealed to different features of microfilariae that mean these larva may belong to different species of the filarial nematode. In Iraq, only one serological study recorded human infection with *Dirofilaria immitis* by Alia *et al.*, (2013).

Comparing the results of the current study with the key features of microfilariae according to WHO, (1997):

1. Length between 177- 230 (220) μm long, 5.0-6.0 μm width, head space, sheath stains, terminal and subterminal nuclei. Tail tapered, terminal and subterminal nuclei widely separated, fig. (1 and 6)*Brugia* sp.
2. Length between 163-203 (183) μm long, 3.0-5.0 μm width, Small size, long slender, tail aperiodic, fig. (2 and 8)*Mansonella* sp.
3. Length between 244-296 (260) μm long, 7.5- 10.0 μm width, a huge size, Short head space, dispersed nuclei, body in smooth curves. Tail tapered, anucleate, fig. (4 and 10)*Wucheraria* sp.

As noted from the comparison that the current study recorded three genera of filariae according to the morphological characteristics. We recommend that genetic studies be carried out to confirm these results.

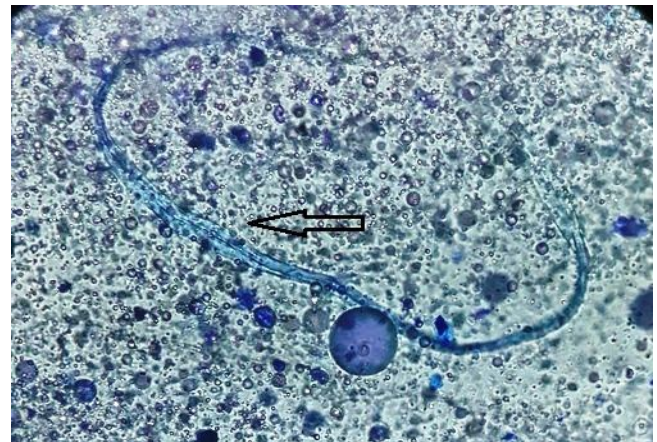


Fig. 10: Large size of microfilaria in blood human stained with Methylene blue by Knott technique, 10X.

Conclusions

The current study revealed to 35.92% rate of infection with filariasis in Iraq that risk and dramatic result must take consideration and applied eradication programme from WHO. All the ages and both gender were infected with filariasis in Iraq. A modified Knott technique was the best for diagnosis of Microfilaria; All of the stains were applied in current study appeared positive rates for infection (Methylene blue, Giemsa and Lieshmen). This study recorded three genera of filariae according to the morphological characteristics (*Brugia*, *Mansonella* and *Wuchereria*), so we recommend that genetic studies be carried out to confirm these results.

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