Development of a simple and rapid method to confirm chyle in biological fluid: Short Communication

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Abstract

Chyle consists of lymph and emulsified fats absorbed from the intestine after digestion and absorption. Patients are often found of chyluria in endemic parts of Africa, India and southern Asia It is caused by the parasitic filarial nematode Wuchereria bancrofti (W. bancrofti). We often receive body fluids mixed with chyle eg. chyluria, chylothorax, chyloperitonium etc. in laboratory for confirmation. Well established biochemical method is there, but it demands expertise and time. To establish a simple method to confirm chyle in biological fluid .Equal volume of suspected chyle sample and ether are mixed by vigorous shaking and standing for 5 minutes, a white band appears at the junctions of two liquids. Sample taken from white band and observed under microscope. Round retractile globules can be seen. Utility of our method is that it does not demand too much of expertise, very short procedure and this method can be used in field where filariasis is endemic.

Keywords: Chyle; Ether; Rapid.

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Chyle consists of lymph and emulsified fats absorbed from the intestine after digestion and absorption. The amount of dietary lipids after digestion and absorption are transported via the lymphatics,15 and 72% of the body's lymph is drained by the thoracic duct **(Udonsi, 1986)**.

Presence of chyle in biological fluid (urine, pleural fluid, peritoneal fluid, etc.) gives white opacity and intensity of white opacity depends on concentration of chyle. Slight white colour fluid has to be confirmed for presence of chyle. Chyle contains chylomicron (a lipoprotein) which gives white opacity. Cavity of chylomicron contains triacylglycerol (fat) and very small amount of cholesterol ester. Patients are often found with chyluria in endemic parts of Africa, India, and southern Asia It is caused by the parasitic filarial nematode *Wuchereria bancrofti* (*W. bancrofti*). Established qualitative biochemical test exist to confirm chyle. The method takes long time and expertise also. In expertise hand, false negative results are encountered **(Wittke,1983).**

The research followed the tenets of the Declaration of Helsinki. Informed consent was obtained from the subjects after a detailed explanation of the nature and possible consequences of the study. This work has been sanctioned by "Institutional Ethics Committee" A total of 56 suspected chyle samples were tested by the biochemical method (to the sample Lipase enzyme, bile salt and phenolphthalein indicator are mixed. 0.1M sodium bicarbonate solution is added drop by drop slowly with continuous shaking. When slight pink colour develops, addition of the solution is stopped. It is then kept at 37ºC**.**

If chyle is present, triacylglycerol is hydrolysed liberating free fatty acids which shift the pH to acidic side and pink colour of phenolphthalein disappears. This confirms presence of chyle) as well as by our method.

Modified simple and rapid method Procedure: About 1.0 ml suspected chyle sample (**Fig.1**) is taken in a 12mm x 75mm small transparent glass tube and equal amount of ether is added **(Richard et al., 2021)**. The tube is mixed vigorously for one minute capping the upper open side and allowed to stand for 5 minutes for phases to separate.

On standing, homogeneous white opacity disappears and a white band appears at the junctions of two liquids. (**Fig.2**).

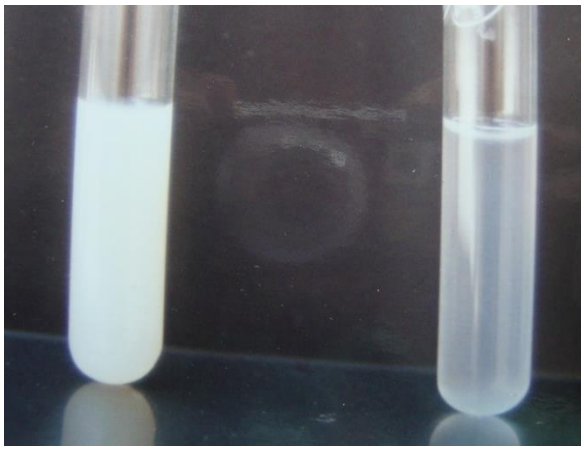

Fig.1. Chyle sample- Chyluria (left) and Chylothorax(right)

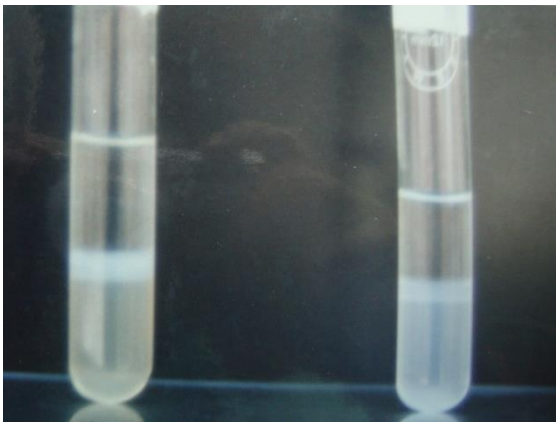

Fig.2. Two different Chyle samples after treating with ether

By a micropipette, the liquid from white band is taken carefully and one drop is put over microscope slide. Now, the fluid under cover slip is examined under microscope **(Austin ,1901)**.

A total of 56 suspected chyle samples were tested by the biochemical method as well as by our method. Biochemical method confirmed 41 samples and our method also confirmed same 41 samples. Out of 15 negative samples, 05 samples were of chylothorax and chyloperitoneal fluid. Slight white opacity was due to high protein content. Rest 10 samples were of suspected chyluria and these were slightly opaque. Out of these, 04 samples had high phosphate content and rest 06 samples were of high bacteriuria i.e., none of the positive samples were missed by our method. If round refractile globules are seen, this confirms fats i.e., presence of chylomicrons in the sample (**Fig.3**).

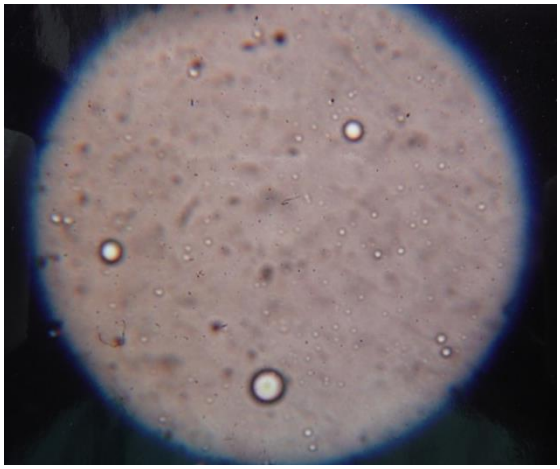

Fig.3. Refractile fat globules of different sizes

Chyle contains chylomicrons which give white opacity of fluid. It is a lipoprotein containing mainly dietary triglyceride and small amount of cholesterol ester. In filariasis, lymphatic channels are blocked by parasites, followed by inflammation and fibrosis of lymphatic channels takes place. Proximal to block, the lymphatic channels burst by lymph pressure, and lymph may be found in urine, pleural fluid,peritoneal fluid, etc **(Alleman ,2003).**

When chyle containing fluid is vigorously shaken with ether, the hydrophobic phospholipid single layer membrane of chylomicron is stripped off by ether and micro-fat globules are liberated. These micro-fat globules coalesce to form bigger globules by hydrophobic interaction which then visible under microscope as refractile globules.

Utility of our method is that it does not demand too much expertise, very short procedure, and this method can be used in field where filariasis is endemic. Many endemic zones are there in India.

This is a very simple and rapid procedure to confirm chyle in biological fluid. The procedure does not require too much expertise. The method can be adopted in field visit where filariasis is endemic.

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