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Isolation of *Mycobacterium tuberculosis* from cerebrospinal fluid by the centrifugation & filtration methods

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Cerebrospinal fluid (CSF) samples were collected in 2 bottles each, from 112 children, examined clinically for tuberculous meningitis (TBM). One was processed by the centrifugation method and the other by the filtration method for the isolation of *M. tuberculosis.* Of these specimens, 11 and 13 yielded *M. tuberculosis* by the centrifugation method and the filtration method, respectively. In 7 specimens *M. tuberculosis* was isolated by both the methods; in 4, only by the centrifugation method, and in 6, only by the filtration method. Using both the methods, 17 (15.2%) of 112 specimens were culture positive for *M. tuberculosis.* The improvement in the rate of isolation, thus obtained, assumes importance as the confirmation of the diagnosis of TBM in all the clinically suspected cases is always desired. Moreover, the filtration method is simple and inexpensive and it can be carried out even in remote hospitals and the membranes, after filtration, can be transported to central mycobacteriology laboratory for culture of tubercle bacilli.

Key words CSF - filtration method - *Mycobacterium tuberculosis* - tuberculous meningitis

Meningitis, due to *Mycobacterium tuberculosis* is known to cause very high mortality, especially in children[1]. The diagnosis of tuberculous meningitis (TBM) by the demonstration of acid fast bacilli in cerebrospinal fluid (CSF) smears is rare and isolation by culture is only between 30 to 60 per cent in most of the studies[2,3]. Therefore, there has always been a need to develop more sensitive culture methods in order to confirm the diagnosis in as many cases as possible.

It is known that the tubercle bacilli has the tendency to float up in body fluids having higher specific gravity[4]. Hence, it is presumed that the tubercle bacilli float up in the CSF during centrifugation and the rate of isolation in the centrifugation method is low. If this is true, filtration of CSF through a filter membrane and processing of the membrane for culture can be expected to yield more culture positives. Moreover, in the centrifugation method that is being practiced, the isolation of *M. tuberculosis* involves inoculation, onto four different culture media, of each of the untreated and the sulphuric acid treated CSF deposits[5]. As this procedure is laborious and expensive, it is desirable to evolve a simple and inexpensive procedure for the isolation of *M. tuberculosis* from CSF samples. Therefore, the objective of the present study was to determine the rate of isolation of *M. tuberculosis* by the centrifugation and filtration methods separately from aliquots of the same CSF specimens.

Material & Methods

CSF specimens : The CSF specimens from 112 children, investigated for TBM at the Institute for Child Health, Madras, were studied. From each of these patients, the specimen was collected in 2 bottles and

transported without delay to the Tuberculosis Research Centre, Madras for culture of *M. tuberculosis.* One was processed by the centrifugation method and the other by the filtration method.

Centrifugation method[5] *:*

Culture by direct inoculation—One loopful of untreated CSF was inoculated onto each of the three solid media *viz.*, Lowenstein-Jensen medium (LJ), LJ with pyruvate (LJP) and selective Middlebrook 7H11 medium (selective 7H11) and 0.2 ml was added to the selective Kirchner' medium (SKLM) using sterile pipette.

Inoculation after decontamination—After direct inoculation, the remaining CSF was centrifuged at 3000 rpm for 15 min. To the deposit 1 ml of sterile distilled water was added followed by 1 ml of 5 per cent sulphuric acid. The bottle was shaken well and kept for 15 min. Five ml of sterile distilled water was added to this and the contents centrifuged at 3000 rpm for 15 min. To the deposit 0.2 ml of sterile distilled water was added and one loopful each was inoculated onto 3 solid media; the remaining inoculum was transferred to SKLM.

Culture reporting—The specimen was reported positive for *M. tuberculosis* if growth was found in any one of the four media used, negative if there was no growth on any one of the media up to 8 wk, and contaminated only if all the four media were contaminated. The growth in the SKLM was confirmed by subculture on LJ.

Filtration method : CSF specimen was filtered, using a syringe, through a membrane filter (Pore size: 0.45 micron; diameter : 25 mm; from Advanced Micro devices Pvt. Ltd., Ambala, India) assembled in a filter holder (Laxbro, India). The membrane filter was then transferred to SKLM and incubated at 37°C. The SKLM bottles were examined and processed for culture as described above.

Results & Discussion

The culture results of the 112 specimens, obtained by the two methods, are presented in the Table. Of these specimens, 11 and 13 were positive on culture by the centrifugation method and the filtration method, respectively. In 7 specimens, *M. tuberculosis* was isolated by both the methods; in 4, only by the centrifugation method, and in 6, only by the filtration method. When both the methods are employed 17 of 112 (15.2%) specimens yielded *M. tuberculosis.* We found (data not shown) that 11 of the 29 culture positive specimens yielded *M. tuberculosis* when their supernatant fluid was filtered and the membranes were processed for culture, clearly suggesting the tendency of the tubercle bacilli to float up in the CSF during centrifugation.

Table. Culture results of 112 CSF specimens each collected in 2 bottles and processed separately by the centrifugation and filtration methods

		Centrifugation method			
	Culture results	POS	NEG	CONT	Total
Filtration method	POS	7	6	0	13
	NEG	3	81	0	84
	CONT	1	13	1	15
	Total	11	100	1	112

POS, positive; NEG, negative; CONT, contaminated

Using the centrifuged deposit of the undecontaminated CSF for culture on LJ slopes, Verma *et al* [6] were unable to isolate *M. tuberculosis* in 21 specimens. However, using similar inoculum and 2 LJ slopes, one of them contained 2 ml of modified Besredka's medium, Kennedy and Fallon[7] isolated *M. tuberculosis* in 83 per cent of the samples from 52 patients. The yield of cultures in other studies has been very 1ow[3]. Radhakrishna and Mathai[8] suspended the centrifuged deposit in 200 µl of sterile phosphate buffered saline and inoculated this on to LJ slopes. They isolated *M. tuberculosis* in 6 to 52 lumbar CSF, 7 of 8 cisternal CSF and 6 of 8 ventricular CSF specimens. Using multiple media Paramasivan *et al* [5] had shown the isolation rate to be 11 per cent in routine diagnostic work.

The number of cultures contaminated in the filtration method was 15 compared to only one in the centrifugation method. It needs to be pointed out that the final results in the centrifugation method are based on the observation of growth on 10 slopes compared to only two LJ slopes in the filtration method. In addition, the culture details of these 15 specimens revealed that except 2 specimens all the other 13 grew contaminants on one or more culture media in the centrifugation method, implying the bad

quality of the specimens. Therefore, by ensuring stringent measures in the collection and handling of the CSF specimens the rate of contamination can be minimised.

The filtration method is a simple procedure compared to the centrifugation method. It is less expensive, as only one SKLM and one pair of LJ slopes are used compared to one pair each of LJP, selective 7H11 and SKLM, and two pairs of LJ used in the centrifugation method. The filtration method can be done in clinics/hospitals, situated in distant places, and the membrane transported, in SKLM, to a mycobacteriology laboratory for further culture studies. Since lumbar puncture cannot be performed repeatedly, the CSF specimen, once collected, needs to be utilised for as many investigations as possible without jeopardizing the results. When the volume of CSF collected is small, as happens often, the application of filtration method would result in recovering the entire CSF filtrate which would be useful for other investigations. Whereas in the centrifugation method, with such specimens, the volume of CSF remaining would be very little after direct inoculation and centrifugation. The improvement in the rate of isolation in the filtration method assumes importance as it is always desired to confirm the diagnosis in as many cases of clinically diagnosed TBM as possible. To the best of our knowledge this is the first attempt to isolate tubercle bacilli from CSF by the filtration method. The findings of this study suggest that the filtration method can be pursued in the mycobacteriology of TBM.

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