

Medico-Legal Aspects of Microscopic Findings in Drowning

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Abstract

Background: In this study we wanted to find out the relevance of microscopic evidence of foreign bodies in lungs in case of drowning and determine the proportion of cases in which diatoms in bone marrow and those in the drowning medium were identical.

Materials and Methods: This was a descriptive study conducted among 100 cases in the Department of Forensic Medicine, Govt. Medical College, Kottayam.

Results: Striking feature which was brought out from this study was that when light microscopy found foreign bodies in distal air passages and alveoli in only 22% cases, polarised microscopy could do the same in 34% cases.

Conclusion: Polarised microscopy is important in the diagnosis of ante-mortem drowning. Though we always insist of finding diatoms in bone marrow which are identical to those in the drowning medium not only as a diagnostic tool but also as a pathognomonic feature of recognising the drowning medium, this finding was present only in 9% of cases. This suggests the low specificity & low sensitivity of the test and hence this particular test cannot be relied upon at all if it turns negative.

Keywords: Medico-Legal Aspects, Microscopic Findings, Drowning

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Introduction

The postmortem findings in cases of death by drowning are frequently inconclusive. The bodies of murdered persons are frequently thrown into river or lake with the hope that when the body is found, death will be attributed to accidental drowning. If death has resulted from drowning and if an autopsy is performed before postmortem changes have progressed too far the systemic effects of asphyxia will usually be recognized. [1]

Diagnosis of drowning in bodies recovered from water has always caused a great problem to the forensic pathologist. Death by drowning is a diagnosis made based on history, circumstances surrounding death and meticulous autopsy to exclude other causes of death. [2] There is no objective morphologic finding which is pathognomonic of drowning. The pathologic changes revealed at autopsies are often not conclusive in establishing the cause of death as

drowning. The morphological changes are so highly variable from case to case, even under identical conditions that none of them can be completely relied on. [3] Forensic pathologists are in a more difficult situation when the body is in a decomposed or in a mutilated state. In such cases, no accurate conclusion can be drawn from the observed changes. In such circumstances it has been presumed as drowning for reasons that the body has been recovered from water and there is no evidence of any other forms of death. [4] This is of course, is likely to have serious consequences, for it may result in civil injustice of making a wrong diagnosis or that the crime becomes unrecognized.

The inconclusive autopsy findings in death from drowning have urged pathologists to undertake laboratory investigations so as to enable them to diagnose drowning by positive results. Different authors have conducted extensive studies based on the principles of diffusion of water and electrolytes between the alveoli and pulmonary capillaries and its sequelae. Various investigative procedures like study of blood biochemistry, [5] measurement of electrolytes in pleural fluid, blood levels of serum strontium and fluorine, [6] atrial natriuretic peptide etc. have been tried for solving this problem.

Another important development in the diagnostic methods of drowning is the detection of diatoms in the lung, bone marrow and other internal organs. Even the diatom test is considered as gold standard by many, even this test has fallacies. Presence of foreign bodies, which are constituents of the drowning medium, in the respiratory passages has always been regarded as a significant finding even in decomposed bodies in the diagnosis of ante-mortem drowning. But they are also known to enter the respiratory passages post-mortem at least to some depth. [7] Hence a study is very much relevant to find out gross and microscopic findings in drowning that will aid in establishing a definite diagnosis of ante-mortem drowning, incorporating its merits and limitations.

Aims and Objectives

1. To find out the relevance of microscopic evidence of foreign bodies in lungs in case of drowning
2. To identify the proportion of cases in which diatoms in bone marrow and those in the drowning medium were identical

Materials and Methods

This was a descriptive study conducted among 100 cases in the Department of Forensic Medicine, Govt. Medical College, Kottayam.

Study Procedure

Bits of parenchyma of lungs beyond the smallest division of bronchi already exposed were collected and preserved in 10% formalin and properly labeled. After fixation the bits were processed and sections of 5 to 7 microns thickness were prepared for microscopic examination for evidence of foreign body. The slides were examined under low power and then under high power microscope. Later the slides are examined under polarizing microscope.

Procedure for Detection of Diatoms from Water Sample and Bone Marrow

Water from the site of drowning is collected in a 2 liter capacity bottle. About 15 ml of iodine solution is added and allowed to settle overnight, this will kill the other micro-organisms. The bulk of water is decanted and the remainder is centrifuged at 3000 revolutions per minute for 5 minutes to recover diatoms. The deposit is placed on a glass slide and a cover slip is placed and viewed under microscope.

Five to ten grams of bone marrow is put in a test tube and covered with conc. Nitric acid about 5 times its volume and left at room temperature for 1 to 2 days for digestion of organic material. The tube is centrifuged and supernatant disc of fat is poured off. The fluid left behind is diluted with distilled water and centrifuged at 3000 revolutions per minute for 5 minutes. The whole process is repeated 2 to 3 times and the deposit which

is hardly visible to naked eye is examined under microscope.

Polarizing Microscope

In ordinary light, vibrations occur at various angles (or axes of vibration) around the principal direction of the beam (while perpendicular to the direction of beam) so that resultant wave motion occurs in all different planes along the principal line of propagation. In plane polarized or linear polarized light, the vibration is limited to only one axis. Therefore the wave is limited to one plane, the plane of vibration.

The polarizing microscope by making use of plane polarized light rather than ordinary light, brings in to the field of observation all of the common properties of anisotropic materials which include several crystal substances such as birefringence. The foreign bodies like sand detected in the alveoli and air passages are crystalline and can show these properties and hence can be observed through polarizing microscope.

In crystals the molecules are arranged in a regular lattice structure and exhibit marked effects on the light transmitted through them. These crystals show the property of birefringence or anisotropism or double refraction or the property of exhibiting different refractive indices towards light passing through the crystal. Anisotropic

substances also polarize the light passing through them. All crystals are readily distinguished from non-crystalline or isometric materials by using polarizing microscope.

When viewed under polarizing microscope quartz or sand will alternately appear black with a succession of bright colours. Even the untrained person can use it to great advantage and if properly performed it is entirely conclusive.

Statistical Analysis

The data obtained from the study is entered in MS Excel and further analysis is done using the software SPSS 16.0 version software with respect the proportion of drowning cases with foreign bodies under light and polarizing microscope and correlation of diatoms in bone marrow and drowning medium.

Results

The study sample consisted of mostly males (85%) as compared to females (15%).

21% of the cases were suicidal, 78% were accidental and one case was homicidal.

Distribution of Co-Morbidities

The distribution of co-morbidities in the study sample is as follows.

Table 1

Co-Morbidities	Frequency
Nil	62
Chronic Alcoholism	19
Psychiatric illness	9
Seizure Disorder	6
CAD	2
Hypertension	1
Chronic Kidney Disease	1

No subjects gave a known history of diabetes mellitus. Omitting those without any co-morbidities, we can clearly see that chronic alcoholism is the highest known co-morbidity among those who drowned (50%). Among the other half 48% of cases suffered from psychiatric illness and 32% of cases suffered from seizure disorder – all

suggestive of CNS disorders. The study showed that among those who had co-morbidities who died due to drowning, majority (50%) was alcoholism while all other co-morbidities were 25% or less. In the current situation of Kerala, where the habituations are moving from smoking more towards alcoholism, which is well evident by

the regular reports of amount spent on alcohol by the beverage corporation of Kerala during festival seasons, this finding definitely highlights the importance of control of social disasters like alcoholism in prevention of death due to drowning.

Level of Air Passages up to which Foreign Bodies are Present

Among those who had foreign bodies in air passages (25 cases), 21 showed the foreign bodies in or beyond the third division of bronchioles while in 4 cases it was present only upto the second division.

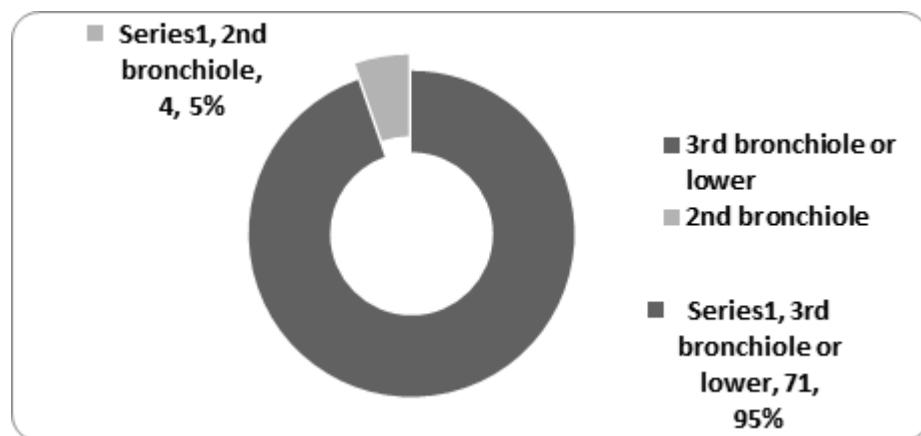


Figure 1

Presence of Foreign Body in Distal Bronchioles & Alveoli as Found out by Microscopy

Light microscopy could find out foreign bodies in distal bronchioles & alveolis in 22% of cases while polarised microscopy

could do the same in 34% of cases. This clearly shows that polarised microscopy is superior to light microscopy in identifying foreign bodies in the distal parts of breathing mechanism. This could help in better identification of antemortem drowning cases from postmortem cases.

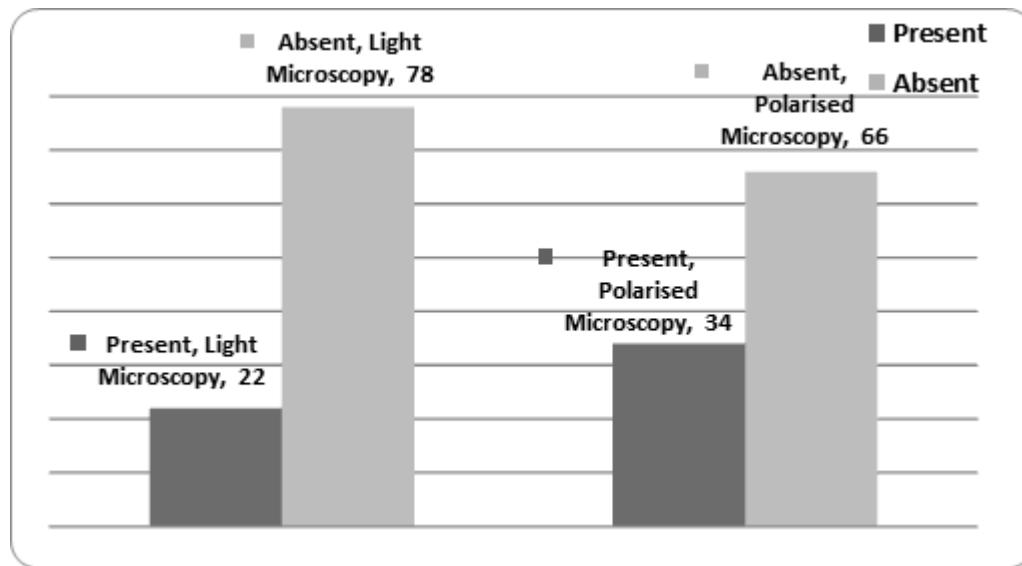
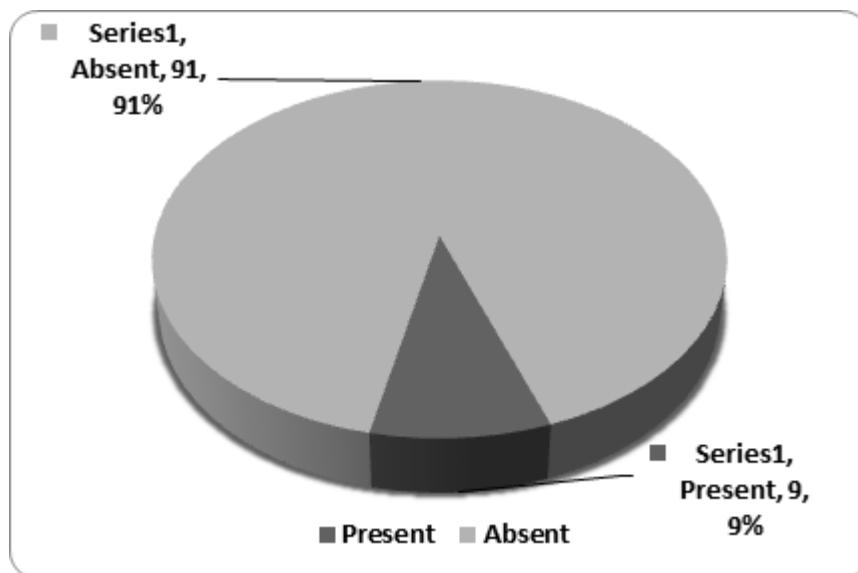


Figure 2

Distribution of Diatoms in the Study Sample

Diatoms were present in all the water samples analysed; but diatoms in bone marrow was found identical with that in the drowning medium only in 9 cases (9%).

**Figure 3**

All through the years we had been highlighting the importance of diatoms in the diagnosis of death due to drowning. It was always highly expressed that finding diatoms in bone marrow which was identical to diatoms found from the medium of submersion suggests that the death occurred in that particular media itself. The investigator although accepts this fact, this study had brought into daylight that the chance of getting identical diatoms in bone marrow to that of the drowning medium is very less (9%). It may be due the small amounts of bone marrow examined in case of usual postmortem examinations.

Discussion

Microscopic examination of lung tissues does not yield much to the diagnosis of drowning. Ordinary stained hematoxylin and eosin sections show distension of the alveolar walls. Reh applied the silver impregnation method of Gomori to the lung sections; thereby revealing the reticulin fibre structure. Based on the degree of distension of alveolar walls he distinguished four types. [8]

- 1) The thickness of the alveolar walls (normally 2 to 3 times the capillary width) is reduced to capillary width. The capillaries are rounded or oval lying in an orderly pattern like the links of a chain. Partial fibre rupture may be observed.

- 2) The alveolar walls are more distended. The capillaries lie separately and their oval lumina are diminished. Fibre ruptures are distinctly observed.
- 3) The distension of the alveolar walls is maximal, the capillaries appear thread like, and only occasional lumina are observed. Distinct intraseptal fibre ruptures are observed.
- 4) This stage is seldom seen and may involve scattered alveoli. There is complete rupture of the wall with retraction of fibres which appear broad and wavy, sometimes like a cork screw.

The microscopic appearance reflecting the spectrum of macroscopic appearances varies from being suggestive of drowning to entirely normal. These findings are however only of significance in the diagnosis of drowning if the following conditions can be ruled out like the aspiration of blood, capillary bronchitis and fatal asthmatic attack, hypoxia from smothering or strangulation and chronic emphysema. The exclusion of chronic bronchitis and asthmatic attacks may not be possible without a good history. Microscopy is necessary to rule out underlying diseases not visible at autopsy, that contributed to death but not useful in establishing drowning as a cause of death. [9]

According to Joshua A Perper and Cyril H Wecht microscopic examination of lungs has

disclosed no findings of sufficient specificity to allow diagnosis to be made with certainty, however if consideration is made of the gross pulmonary findings and the circumstances of death, the diagnosis of drowning can be made with reasonable degree of certainty.

The presence of foreign particles in the alveoli can be demonstrated by light microscope and can be confirmed by using Polarizing microscope. Sand particles are crystalline particles with orderly arrangement of their molecules and are anisotropic substances with many unique optical properties. They exhibit the property of birefringence; they exhibit different refractive indices towards light passing in different directions through the crystals. When viewed in polarized light and the stage is rotated it will alternately be black and a succession of bright colours will occur. [10]

Diatoms in Drowning

Detection of diatoms in the bone marrow and internal organs such as lung, liver and kidney is considered a good diagnostic aid in case of drowning. A number of studies have been undertaken on the subject by various authors. [11]

Diatoms are unicellular algae found wherever there is water and enough light for photosynthesis. In wet type of drowning these diatoms also enter the lungs along with water. They also absorbed in to the blood and reach the various internal organs. It was Revenstorf (1904) who showed convincingly that microscopic particulate matter present in the fluid medium could penetrate the peripheral regions of lung in drowning. He detected the plant materials in the fluid expressed from lungs in case of drowning. These particles known at the time as planktons consist of algae, diatoms and a variety of microscopic debris. [12]

The diatoms are present in natural water ponds, rivers, lakes or any place where there is stagnant water. There are countless individual species of them. The number of diatoms present in the water varies according to the season of the year.

Characteristics of Diatoms

About 15000 different species of diatoms have been discovered so far in which half are found in fresh water and half in sea water. The sea water diatoms are more fragile than the fresh water one because they contain less silica. Diatoms live free as individual particles or colonies. They may be present along with other planktons and exist in the surface of water. The colonial picture is due to their aggregations formed by union with a mucous like secretion. Adherence of one to other is also possible.

The cell structure of diatom is unique in that they secrete a hard siliceous capsule called frustule which is chemically inert. [13,14,15,16] They vary in size and shape. They may be circular, elliptical, triangular, square, hexagonal, rectangular or any other geometrical pattern. Each diatom has got a double wall and cross-striae or bands and are resistant to heat or acids. This forms the basis for their isolation from organic materials even after putrefaction. The silica particles render them resistant to physical or chemical injury.

The size of the diatom varies according to species. They vary in size from 2 microns to 1 millimetre, the usual size being 10.8 microns, with varying widths. Some of the diatoms are motile and others non motile. They come under the phylum Heterokontia. They are further subdivided in to three classes which is the most recent classification as proposed by Crawford and Round in 1990.

- Coscinodiscophyceae (radially symmetrical)
- Bacillariophyceae (bilaterally symmetrical with raphe)
- Fragilariphycace (bilaterally symmetrical without raphe)

Drowning is one of the common causes of unnatural death in India especially in the coastal states like Kerala, Tamilnadu, Karnataka, Maharashtra etc. The drowning can be accidental, suicidal and homicidal. Accidental drowning is common.

The study about the presence of identical diatoms in bone marrow and water sample in drowning and non-drowning cases is helpful to assess the reliability of diatom test in drowning cases and for a better diagnosis.

Studies about diagnosis of drowning by utilizing the presence of diatoms was available since 1904(Revenstorf-Medi-Sci-Law-1980) but Gay (1861) described the appearance of drowned subjects. He observed that the trachea and larger bronchial tubes contain water which sometimes penetrates to their most minute ramifications. Brouardel and Vibert (1880) conducted a series of experiments on drowned dogs. They showed that death by prolonged submersion a quantity of water equal to a third or quarter of the subjects blood volume enters the circulation. Other eminent persons who conducted studies on diatoms and drowning were Swannand Spafford (1951) Crossfill (1955), Rushton(1961),Thomas, Vanhke and Timperman (1961-1963), Timperman (1969,1972), Gertson(1977), Giri(1993), Mattusumoto (1993), Ludes (1993), Pollanen (1997,98) and Lunetta (1998).

Lunetta.P. of Helsinki, Finland (1998) studied the scanning and transmission electron microscopical evidence of the capacity of diatoms to penetrate the alveolo-capillary barrier in drowning. The transmission electron microscopy analysis shown that the diatoms in the alveolar spaces and detected their phagocytosis by alveolar macrophages and the diatom entered the interstitial spaces and to pulmonary capillaries and to venules.

The Common Type of Diatoms

1. Epithemia (Rectangular in shape with double wall and stroma of fine radiating pattern.)
2. Nitzchia (Narrow rectangular shape)
3. Cymbella (Saucer shaped)
4. Raphoneis amphicerous (Spindle shaped)
5. Amphicerous elongate (Long and narrow spindle shaped)
6. Segmented (Ladder type)

7. Try radiating.
8. Cyclotella (Rounded)
9. Star shaped
10. Milosira juergensu
11. Eunotia otiamandon
12. Pinnularia viridis.

The cell structure of diatom is unique in that they secrete a hard siliceous capsule called frustules which is chemically inert, almost indestructible being resistant to strong acids.[17] During drowning smaller diatoms present in the drowning medium enter systemic circulation, having passed through the filter of lungs and become lodged in tissues such as the bone marrow, liver, kidneys, and brain where they can be demonstrated. Only a live body with intact circulation and a beating heart can transport the diatoms to distant organs.

Tedeschi has described four methods for demonstration of diatoms:

1. Direct microscopic examination: This is used only in the examination of lungs. Water is squeezed out from the lungs, centrifuged and the sediment is examined.
2. Microscopic examination of tissue sections by the method of Weining and Pfanz where by optically empty sections of diatoms are produced.
3. Chemical digestion the tissue is treated with stron acid whereby all organic matter is digested, but the silicaceous capsule of diatoms is well preserved.
4. The organic matter is destroyed by incinerating in an oven.

A recent method for detection of diatoms by enzymatic digestion with protienase K has been proposed by Ludes B, Quantin et al and found useful in decomposed bodies.[18]

Procedure for Detection of Diatoms in Water Sample

It was described in detail by Ingram and Hendey. Water from the site of drowning is collected in a 2 liters capacity bottle. 15 ml of lugols iodine solution is added and allowed to settle overnight, this will kill the other microorganisms. The supernatant

solution decanted without disturbing and the remainder taken in a centrifuge tube and centrifuge in an electric centrifuge in 3000 RPM for 5 minutes, to recover diatoms. The supernatant solution is decanted and the filtrate taken in a glass slide and a coverslip and examined under a light microscope with 10 and 45 times magnifications. [19]

Procedure for Detection of Diatoms from Tissues

This procedure was described by Thomas and his associates. Bone marrow is probably the tissue of choice. [20] One or more long bones are used; usually femur is the long bone of choice. [21,22] Sternal bone marrow can also be taken according to Krishnan. All soft tissues attached to the bone are removed and the bone is scrubbed thoroughly. Half way along the shaft for a distance of 2cm a layer of bone is cut out for a depth of 2mm with the help of a machine tool. This procedure prevents contamination of bone marrow. The shaft is then sawn through at the sites marked out by the machine tool and the bone marrow collected using a gynaecological curette of adequate size. Thomas suggests that approximately 15 to 40 grams of bone marrow can thus be removed. [23] The marrow is placed in a Kjeldahl's flask in which it is chemically digested by adding concentrated Nitric acid about five times its volume, adding small quantities at a time and then heated. Sulphuric acid is contraindicated because it produces precipitates obscuring the microscopic picture completely. But authors like Apurba Nandy and Mukherjee advocates the use of sulphuric acid as it carbonizes the organic material. This procedure yields a transparent fluid with a supernatant disc of fat. The yellow fluid is next centrifuged 2 to 3 times and the deposit usually hardly visible to naked eye is poured on a glass slide and examined while still wet under cover glass or can be mounted in a medium with high refractive index.

While doing diatom test two requirements should be met before this test can be accepted namely that all species recovered from an organ must be present in samples of water

taken from the place of submersion of the body and they must be present in the same proportions in the organs as in the site sample. [24] Obviously these requirements are not easy to meet.

Proponents of the diatom test emphasize that the demonstration of diatoms may confirm the following

- That death was due to drowning
- The person was alive when he entered the water
- The site of drowning by comparing diatoms in water and body.[25]

Timpermann made thorough studies of the test and concluded that it provides the most reliable proof of drowning. Experts like Thomas Van Hecke have showed that the method is reliable if adequate precautions are taken during the procedure to avoid contamination. But others like Spitz and Schneider, Schmidt and Fett found diatoms in 96% of non-drowning cases. [26]

Hendey reviewed the diatom test from a biologist's point of view. He commented on the two facts that diatoms may be absent in organs of persons who are known to have Drowned and diatoms may be present in persons not dead from drowning. With regard to the first fact one must take in to account the seasonal variations in the population of diatoms in to consideration. If a person drowns during a period when the water is almost devoid of diatoms, the search for diatoms in his organs may be futile, no matter how much water has been inhaled.

The second fact may have three explanations.

- Diatoms may be present in raw vegetables, fruits and shell fish which we eat; they may somehow enter the lungs and the distant organs from the stomach.
- Large amounts of diatomaceous earth (Kieselguhr) are used in the manufacture of building materials, paints, paper etc. which may be inhaled along with air and are carried to distant organs.
- Certain dusting powders may also contain diatoms which may be inhaled.

Nanikawa and Kotoku describe cases where diatoms were found in bodies' dead from other causes and buried at a depth of 120 metres in sea where they might have reached due to the effect of hydrostatic pressure. Also, Karkola claim that diatoms and other dirt particles migrate to the left side of heart after death.

So while doing diatom test adequate precautions should be taken during each step to avoid contamination of samples. Most pathologists do not believe that the laborious and technically difficult diatom testing is sufficiently specific or sensitive for the diagnosis of drowning. [27,28]

Conclusion

Striking feature which was brought out from this study was that when light microscopy found foreign bodies in distal air passages and alveoli in only 22% cases, polarised microscopy could do the same in 34% cases. This clearly suggests the importance of polarised microscopy in the diagnosis of ante-mortem drowning. Though we always insist of finding diatoms in bone marrow which are identical to those in the drowning medium not only as a diagnostic tool but also as a pathognomonic feature of recognising the drowning medium, this finding was present only in 9% of cases. This suggests the low specificity & low sensitivity of the test and hence this particular test cannot be relied upon at all if it turns negative.

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