

## Role of Diatoms in Diagnosis of Death Due to Drowning: Case Studies

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### ABSTRACT

**Background:** The most frequent use of diatoms in forensic science is the diagnosis of death due to drowning. The basic principle of diatom test in drowning is based on the inference that diatoms are present in medium where drowning took place and the inhaled water enters the alveolar spaces of lungs and penetrates from the alveoli into the blood circulation, thus microscopic unicellular algae called diatoms get transported to different organs of body. The diatoms found inside the body of drowned victim may serve as corroborative or even conclusive evidence to support the diagnosis of death. It can be ascertained whether the drowning is ante-mortem or post-mortem.

**Case Report:** The present study was conducted in Biology and Serology Division of State Forensic Science Laboratory, Shimla Hills Junga, Himachal Pradesh, India. Water sample from putative site of drowning as well as hard bones (sternum, clavicle, femur), soft tissue (spleen, liver, kidney) and peritoneal/pleural cavity fluid after post-mortem examination of dead body was sent to the Laboratory for the detection of diatoms. Cases were opened and processed following standard methodology. In the present study, 17 cases of drowning were examined for detection of diatoms. Out of seventeen cases, there were 15 males and 2 females aged between 11-63 years. 12 cases revealed the presence of diatoms (death due to drowning) while 5 were found to be negative (death other than drowning).

**Conclusion:** The present study revealed the importance of diatom test in forensic investigation and diagnosis of death due to drowning. Diatom test proved very significant application in forensic science in solving the drowning cases.

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► *Implication for health policy/practice/research/medical education:* Role of Diatoms in Diagnosis of Death Due to Drowning

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## 1. Introduction:

Drowning is a form of mechanical asphyxia caused by inhalation of fluid into air passages due to submersion of body (nose and mouth) into water or fluid. Diatom, a unicellular alga with a siliceous cell wall have been recorded and classified for over 200 years and have been used in a range of applications in forensic science. Diatoms are found in almost all water bodies. The basic principle of diatom test in drowning is based on the inference that diatoms are present in medium where drowning took place and the inhaled water enters the alveolar spaces of lungs and penetrates from the alveoli into the blood circulation, thus diatoms get deposited in internal body organs like brain, kidney, sternum, femur and other organs (1-4). The types of diatoms found inside the body tissues are compared with types of diatoms species present in water sample from putative site of drowning. The same species of diatoms found inside the body of drowned victim as that of putative drowning medium may serve as corroborative or even conclusive evidence to support the diagnosis of death (5-10). However, if the victim was dead before the body was submerged, any diatom entering the lungs with water would not be transported to distant body organs because of lack of blood circulation. Although, there are some typical signs of drowning known, it is still difficult to ascertain a death by drowning due to absence of typical post-mortem findings in case of highly putrefied and skeletonised bodies recovered from drowning medium and exhumations (7). In such instances presence of diatoms in internal organs becomes the only reliable screening method of knowing whether the death is ante-mortem drowning or post-mortem disposal. The collection of diatoms in victim's bone marrow represents a microbial fingerprint of the time and place where drowning occurred (11). The autopsy diagnosis of death by

drowning can be one of the most difficult problems in forensic pathology because the time required to complete the inquest formalities and transport of body to the mortuary is enough to mask the most of the post-mortem evidences of cause of death (12).

Detection of diatoms from internal organs of victims is considered as positive proof of ante-mortem drowning by the forensic pathologist nationwide (13). Drowning is mostly accidental. In a relatively small number of cases, it is suicidal and in some other cases death in water is due to natural causes such as myocardial infarction (cardiac arrest due to absence of blood supply) or cerebral haemorrhage (bleeding from blood vessels of brain) during bathing or swimming (13). Thus, when a dead body is recovered from water, there is usually a suspicion whether the person was alive or dead before entering the water body.

While solving drowning cases, a positive correlation between diatoms detected from bone/ tissue samples and from putative drowning medium has to be established for successful determination of drowning site (9, 10). Diatoms do not occur naturally in the body. The presence of diatoms in internal organs is a proof that person was alive before entering the water, proving cause of death to be drowning. However absence of diatoms does not immediately rule out drowning, the test does not prove negative and thorough investigation is always required (9, 10, 14, 15). At present, the combination of all autopsy findings and diatom test can provide useful information for tentative diagnosis of drowning and arriving at a conclusion (16). The main objective of this study is to explore the importance of diatom test in forensic investigation and diagnosis of death due to drowning.

## 2. Case Report:

The present study was conducted in Biology and Serology Division of State Forensic Science Laboratory Shimla Hills, Junga, Himachal Pradesh, India and all the cases reported here are of fresh water drowning except one drowning in pool. Water sample

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from putative site of drowning as well as hard bones (sternum, clavicle, femur) and soft tissue (liver, spleen, kidney) after post-mortem of dead body was sent as crime exhibit to the Laboratory for detection of diatoms. Cases were opened and processed following standard methodology (14).

In cases where bones were preserved, the hard bones were longitudinally sectioned using clean band saw and bone marrow was extracted using clean spatula in a beaker. In cases where liver/spleen/kidney were preserved, tissue was collected in a beaker and where peritoneal fluid, pleural cavity fluid was sent for examination, fluid was put in a beaker. 50 ml. of nitric acid was added to the beaker containing bone marrow, tissue, and peritoneal fluid, pleural cavity fluid sample and kept undisturbed for 3 days. Then sample was put on hot plate at 60-70<sup>0</sup>C in a fume hood. More nitric acid was added when it started drying. After three days, fat layer formed at top is removed carefully and remaining sample was centrifuged at 3500 rpm for 10 minutes. The process of centrifugation was repeated 3 times with distilled water. Supernatant was discarded and pellets were washed with distilled water and recentrifuged. Thin smear was prepared by putting residues on slides. After drying, slide was examined under microscope.

Lugol's iodine was added in water sample to inhibit microbes and kept overnight. The Lugol's iodine digested water sample is centrifuged three times at 3500 rpm for 10 minutes and washed with double distilled water. Supernatant was discarded and thin smear was prepared by putting residues on the slides. After drying, slides were examined under microscope. After microscopic observation of diatoms in both samples, a correlation of diatoms present in the water sample in which drowning took place and bone sample was drawn out accordingly.

Out of seventeen cases received for detection of diatoms, there were 15 males and 2 females aged between 11-63 years. The cases are listed in Table 1.

**Case 1.** The body of a male aged 48 years was found floating face down in Renuka lake in the month of September. No signs of

injuries were reported in autopsy. Water sample and sternum bone received in the laboratory for detection of diatoms. After examination, diatoms *Cymbella* sp., *Cyclotella* sp., *Diatoma* sp. and *Epithemia* sp. were detected in bone sample which were comparable with diatoms detected in water sample, proving it a case of death due to drowning which was later proved to be a case of suicide as the deceased was under stress/depression.

**Case 2.** The body of a male aged 26 years who was missing was recovered from Biyut rivulet in the month of August in highly decomposed conditions. The cause of death could not be ascertained in autopsy. Water sample and femur bone were received in the laboratory for detection of diatoms. After examination, diatoms were detected in water sample. However diatoms could not be detected in femur bone. The cause of death was attributed to reasons other than drowning which was later accepted by perpetrators that they murdered the victim and thrown the body in a rivulet to mislead the facts/investigation.

**Case 3.** The body of a male aged 30-40 years was recovered from Lal Pani Nullah (canyon) near bypass Shimla in the month of November in highly decomposed conditions. The cause of death could not be ascertained in autopsy. Water sample and femur bone were received in the laboratory for detection of diatoms. After examination, diatoms *Cymbella* sp., *Aulacoseira* sp. and *Gyrosigma* sp. were detected in water sample. However, diatoms could not be detected in femur bone. Thus, the cause of death was attributed to other than drowning.

**Case 4.** The body of a female aged 36 years was recovered after three weeks from Satluj River in the month of January in highly decomposed condition. No evidence of injuries was found on the body. Water sample and clavicle bone were received in the laboratory for detection of diatoms. After examination, diatoms *Navicula* sp. and *Cocconeis* sp. were detected in bone sample which were comparable with diatoms detected in water sample, proving it to be a case of death from drowning which was later accepted by her husband that he had thrown

**Table 1:** Diatoms detected in water sample and bone/tissue sample of drowning cases.

| Case No. | Sex    | Age (years) | Water sample | Bone sample                            | Diatoms in water sample | Diatoms in bone and other samples | Result   |
|----------|--------|-------------|--------------|--|-------------------------|-----------------------------------|----------|
| 1.       | Male   | 48          | Water sample | Sternum                                | ++                      | ++                                | Positive |
| 2.       | Male   | 26          | Water sample | Femur                                  | ++                      | --                                | Negative |
| 3.       | Male   | 30-40       | Water sample | Femur                                  | ++                      | --                                | Negative |
| 4.       | Female | 36          | Water sample | Clavicle                               | ++                      | ++                                | Positive |
| 5.       | Male   | 17-25       | Water sample | Femur                                  | ++                      | ++                                | Positive |
| 6.       | Male   | 11          | Water sample | Femur                                  | ++                      | ++                                | Positive |
| 7.       | Female | 40          | Water sample | Sternum                                | ++                      | --                                | Negative |
| 8.       | Male   | 42          | Water sample | Femur                                  | ++                      | --                                | Negative |
| 9.       | Male   | 33          | Water sample | Peritoneal fluid, pleural cavity fluid | ++                      | ++                                | Positive |
| 10.      | Male   | 48          | Water sample | Sternum                                | ++                      | ++                                | Positive |
| 11.      | Male   | 45          | Water sample | Liver/spleen / kidney                  | ++                      | ++                                | Positive |
| 12.      | Male   | 63          | Water sample | Sternum                                | ++                      | ++                                | Positive |
| 13.      | Male   | 30-40       | Water sample | Femur                                  | ++                      | ++                                | Positive |
| 14.      | Male   | 55-60       | Water sample | Tibia                                  | ++                      | ++                                | Positive |
| 15.      | Male   | 30-35       | Water sample | Sternum                                | ++                      | ++                                | Positive |
| 16.      | Male   | 22          | Water sample | Sternum                                | ++                      | ++                                | Positive |
| 17.      | Male   | 31          | Water sample | Sternum and humerus                    | ++                      | --                                | Negative |

the deceased in river due to illegitimate relation.

**Case 5.** An unidentified male body was recovered from Bukhari Nullah (canyon) near Shimla in the month of February in highly decomposed condition. The cause of death could not be ascertained in autopsy. Water sample and femur bone were received in the laboratory for detection of diatoms. After examination, diatoms *Navicula* sp., *Cyclotella* sp. and *Diadesmus* sp. were detected in bone sample which were comparable with diatoms detected in water sample proving it to be a case of death by drowning.

**Case 6.** The body of a boy aged 11 years, who was missing since two weeks was recovered from bypass link canal of Khara hydroelectric plant in the month of June in decomposed condition. The cause of death could not be ascertained in autopsy. Water sample and femur bone were received in the laboratory for detection of diatoms. After examination, diatoms *Navicula* sp., *Tabellaria* sp. and *Cocconeis* sp. were detected in bone sample which were comparable with diatoms detected in water sample, proving it to be a case of death due to drowning. During police investigation, deceased's father accepted that he had

thrown the victim in the canal as he suspected his wife of having extra marital affair and child was born out of his spouse's affair.

**Case 7.** The body of female aged 40 years was recovered from Markanda River, Sirmaur in the month of July. As per the information obtained from police documents, deceased was crossing the river when she swept away by sudden gush of water and drowned. In autopsy report, the cause of death was asphyxia due to intense laryngeal spasm (dry drowning). Water sample and sternum bone were received in the laboratory for detection of diatoms. After examination, diatoms *Navicula* sp., *Amphora* sp., *Synedra* sp., *Cymbella* sp. and *Diadasmus* sp. were detected in water sample. However diatoms could not be detected in sternum bone, proving it to be a case of dry drowning.

**Case 8.** The body of male aged 42 years was recovered after four days from the banks of Giri River in the month of July. There was no evidence of injuries on the body. Victim's brother suspected foul play regarding the cause of death. Water sample and femur bone were received in the laboratory for detection of diatoms. After examination, diatoms *Navicula* sp. and *Cymbella* sp. were detected in water sample. However, diatoms could not be detected in femur bone. Thus, the cause of death was attributed other than drowning.

**Case 9.** A male aged 33 years was undergoing swimming orientation training at Nahan in the month of May. He fell unconscious while coming out from the pool. Pool was 15 feet deep. Cardiopulmonary resuscitation (CPR) was given and then victim was shifted to hospital wherein he died of sudden cardiac arrest. Autopsy findings were suggestive of death due to ante-mortem wet drowning. Water sample, pleural cavity fluid and peritoneal fluid were received in the laboratory for detection of diatoms. After examination, diatoms *Navicula* sp. and *Cymbella* sp. were detected in pleural cavity fluid and peritoneal fluid which were comparable with diatoms detected in water sample, proving

the cause of death to be ante-mortem drowning while swimming followed by cardiac arrest.

**Case 10.** The body of a male aged 48 years was recovered from Bhaba River in the month of November in advanced stages of decomposition with partial skeletonisation effect. No evidence of ante-mortem injuries was present on the body. Water sample and sternum bone were received in the laboratory for detection of diatoms. After examination, diatoms *Cymbella* sp., *Gyrosigma* sp., *Cyclotella* sp., *Navicula* sp. and *Amphora* sp. were detected in bone sample which were comparable with diatoms detected in water sample, proving it to be a case of death due to drowning.

**Case 11.** The body of male aged 45 years was recovered from a river near Naina-Balog Theog in the month of November. No evidence of ante-mortem external injuries was present on the body. Deceased was habitual drunkard. Investigations revealed the cause of death as drowning. However, relative suspected foul play. Water sample, liver, spleen and kidney were received in the laboratory for detection of diatoms. After examination, diatoms *Navicula* sp., *Rhoicosphenia* sp., *Synedra* sp., *Diatoma* sp. and *Diadasmus* sp. were detected in liver, spleen and kidney which were comparable with diatoms detected in water sample, proving it to be a case of death due to drowning.

**Case 12.** The body of a male aged 63 years was recovered from underground water tank of under construction building in the month of February in highly decomposed conditions. Deceased was habitual of alcohol and used to sleep near the water tank. No evidence of ante-mortem injuries was present on the body. Water sample and sternum bone were received in the laboratory for detection of diatoms. After examination, diatoms *Navicula* sp., *Cymbella* sp., *Diatoma* sp., *Aulacoseira* sp. and *Diadasmus* sp. were detected in bone sample which were comparable with diatoms detected in water sample, proving it to be a case of death from drowning.

### 3. Discussion:

The diagnosis of drowning remains one of the most difficult issues in forensic medicine. Drowning is substantiated as a cause of death when the types of diatoms in human organs matches diatoms present in the putative drowning medium (1-3) but the reliability and applicability of quantitative and qualitative diatom analysis in the diagnosis of drowning is still controversial (1-3, 17, 18). Certain species are thought to be inhaled either through air (19) or from food and drinking water (20). Few authors have reported the presence of diatoms in bone marrow and tissues of non-drowned bodies (20-23). According to Pollanen, presence of diatoms in non-drowned bodies could be due to contamination caused during various processes of autopsy and diatom test (3, 24). However, there are few studies where no diatoms were detected in tissues of non-drowned bodies (25, 26). Krstic *et al* (2) suggested that rapid death could prevent the penetration of diatoms into the bloodstream and their subsequent deposition in the organs. Indeed, the diatom test is still considered as the golden standard (16). A few authors have strongly supported diatom test in diagnosis of death due to drowning, provided analysis is performed without contamination (3, 24, 27, 28).

In the present study, 17 cases were examined for detection of diatoms. Out of which 12 cases were positive (death due to drowning) and 5 were negative (death other than drowning). Horton *et al*. (29) examined drowning cases on the basis of diatom test. They have developed a quantitative diatom based reconstruction technique to confirm drowning as a cause of death and localize the site of drowning. Li *et al*. (30) examined the diatom quantity in 407 drowning cases. 372 cases revealed positive results from diatom test in lung tissue. Ludes *et al*. (28) examined 4 organ samples of 12 immersion cases for diatoms and diatoms were found to be present in 66% of the lung samples. Kumar *et al*. (6) used diatom test to solve seven drowning cases. Among seven cases of drowning, four were positive and three were negative. Malik *et al*. (8) used diatom

test to solve 5 drowning cases. 3 cases revealed positive results from diatom test. Magrey and Raj (31) examined 31 human drowning cases, 09 cases were positive for diatoms, while 22 cases were found to be negative. Sitthiwong *et al*. (26) used diatom test for detection and identification of diatoms in tissue samples of drowning victims. The diatoms were found in the gastric contents, lungs, duodenum contents, liver, kidney, blood and brain of all twelve of the corpse. Whilst in the tissues of five alternative corpses who died from accidents and diseases as control cases, diatoms were absent. From these different studies conducted in different parts of the World, it can be concluded that diatom test can serve as significant supportive evidence in diagnosis of death due to drowning if proper care is taken to avoid possible contamination. The diatom test due to poor sensitivity is much debated worldwide because it is not possible to rule out contamination. Some water bodies contain only minimal amounts of diatoms which may provide false negative result. Racz *et al*. (32) used PCR based method for identification of drowning cases in which diatom test of post-mortem tissue samples were negative. Samples were tested with algae (diatoms and small green algae) and blue green algae specific primers. In future, PCR based method may be considered as alternative to diatom test in the diagnosis of death due to drowning (32).

### 5. Conclusion:

The most frequent use of diatoms in forensic science is the diagnosis of death by drowning. In the present study 17 cases were examined for detection of diatoms, out of which 12 cases revealed positive results and 5 revealed negative results. The dominant species of diatoms present in water from the spot were comparable with the diatoms found in the samples of each of the drowning victims. Diatom test proved very significant in solving the drowning cases which were in advanced stage of decomposition and in skeletonised bodies and can serve as important evidence to medico-legal

investigation particularly in those cases where autopsy findings and spot findings do not imply drowning as cause of death

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