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Forensic Analysis of Diatoms- A Review

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ABSTRACT

When a corpse is recovered from water, there is usually a suspicion whether it was a case of ante-mortem or post-mortem drowning i.e. whether the body was drowned before or after death. To diagnose the cause of death in such cases, presence of diatoms in the body tissues is very useful evidence. From various chemical and other tests the presence of diatoms inside the body is proved. The type of diatoms found in that body tissues are compared with types of diatoms species present in the water body from where the corpse is recovered to determine the scene of occurrence for the reconstruction of events. By evaluating all these significant aspects, supportive evidence to the conclusion is given. In this present study, an attempt is made to collect the relevant literature and study various methods and techniques used for the detection of diatoms and their evaluation with the aim to find out the best available method and technique on the basis of reliability, sensitivity and reproducibility.

Keywords: Drowning; Diatoms

Introduction

Forensic significance and status of diatom test

Considering the Status of diatom test, British and American forensic pathologists have divergent opinion about the value of diatoms in drowning. Perhaps one of the reasons of this "Great Divide" is that the diatom test originated on the British Continent. There is hardly a medico legal journal that has not taken part in the "war of diatoms" in one way or another. And Forensic Pathology community has been, historically, polarized in its general acceptance of the diatom test as a definitive diagnostic test for drowning.[1] Lack of knowledge and controversy of test makes this test of limited value and raises question about its acceptance.

Diatom test is based on a theory that normally people do not have significant number of diatoms in some important organs like kidneys, brain and bone marrow etc. but if a person drowns in water containing diatoms, the diatoms in that water will reach the lungs and some of them will penetrate the alveolar wall. If the heart is still beating, the diatoms that have entered the blood stream will be transported around the body and may lodge in distant organs such as the kidneys, brain and bone marrow before death. Increase in the number of diatoms in the internal organs was thought to confirm that the body had drowned and, if a sample of the water was also taken at the presumed site of drowning, the similarity of different species of diatoms in the water and the body could be determined. On the other hand, if a dead body was dropped into water, although diatoms could reach the lung by passive percolation, no circulatory transfer could occur and so (theoretically) no diatoms would be present in the distant organs. The advantages claimed for this technique include the fact that it could be used even in the presence of putrefaction, if protected tissue such as bone marrow was examined. Unfortunately, diatom test is often negative, even in undoubted cases of drowning in waterways full of diatoms, and there have been numerous false positive results that are said to have occurred for a variety of technical reasons. Here question arises that why only diatoms are used for this purpose? Some important characteristics of diatoms help them to prove their evidentiary value. Firstly, they are too numerous and more importantly these are the only microorganisms with acid resistant frustules, making them easier to extract from post-mortem tissues. They are too small to penetrate various distant organs of the body. They can be easily classified and identified. Analyses of the concentration of sodium, chloride and magnesium have been used, but the results are too variable (due to the rapid postmortem diffusion of electrolytes through out the body after death) to be of any practical use.

Investigations of homicidal drowning can benefit from the diatom test in two important ways: (1) the confirmation of a diagnosis of drowning made at postmortem examination and (2) the detection of drowning in cases of homicidal deaths involving extensive decomposition or postmortem burning of the body. It is particularly helpful in cases where the body is transported from the putative drowning medium to a dry site on land.[2] The occurrence of diatoms in the bone marrow is a proof that the individual was alive when entered the water. This means that the cause of death was due to the drowning but absence of diatoms does not immediately rule out drowning. Forensic limnology is in critical corporation, and the diatom test is among those modalities that need to remain at center stage. Presence of diatoms in bone marrow, lungs, liver, spleen, kidney, and brain tissue led to the development of the diatom test. This test became a direct screening test for the diagnosis of drowning. The presence of aquatic diatoms in a cadaver has long been held by many workers to be a clear indicator of death by drowning.[2,3,4] The presence of diatoms can be established and analyzed both quantitatively and qualitatively through a diatom test. This can lead not only to a more direct determination of the cause of death, but also can help to pinpoint the site of a suspected drowning.[5]

The Biology and Ecology of Diatoms

Diatoms are unicellular organisms of the kingdom Protista, characterized by a silica shell of often intricate and beautiful sculpturing. Most diatoms exist singly, although some join to form colonies. They are usually yellowish or brownish, and are found in fresh and saltwater (Plate-1), in moist soil, and on the moist surface of plants. They carry chlorophylls *a*, *c* and the carotenoid fucoxanthin present in plastids. They reproduce asexually by cell division. There are more than 200 genera of living diatoms, and it is estimated that there are approximately 1,00,000 species.[6] For the most part

they lack flagella. Although most diatoms are autotrophic, some heterotrophic or symbiotic species can be found in particular habitats. The living matter of each diatom is enclosed in a shell of silica that it secretes. These shells are marked by minute pores or depressions that allow the living organism access to its environment. Diatoms cells are contained within a unique silicate (silicic acid) cell wall comprised of two separate valves (or shells). This cell wall is also called a frustule or test, and its two parts typically overlap one other like the two halves of a Petri dish. When present in abundance in water they impart golden- brown color to that, therefore also known as *Golden Brown Algae*. Diatoms are highly polymorphic on the basis of their size and shapes (Plate-2). They cling together by means of a gelatinous substance to form zigzag rows of ribbon shaped colonies. The diatoms which are motile are often diamond shaped or boat shaped. The characteristic feature of diatoms is their ability to secrete an external wall composed of silica called Frustules.[7]

This fact and the natural competition between equally suited species lead to generic and special variations between and within water bodies. While diatom communities exist almost everywhere, "the communities that live in water under natural conditions usually consist of more species than those communities found in aerial habitats or in the soil".[8] The diatoms are identified by their morphological characteristics. The siliceous exoskeleton imparts uniqueness to their structure. Diatoms are extracted from the various body tissues by various methods of digestion and centrifugation. These should be extracted in such a way that there should be minimum damage to the frustules. These are seen under microscope and compared with the diatoms found in the water samples taken from the suspected drowning area. If it gives a positive result, it can lead towards a positive frame in ascertaining the site of drowning and helps in linking the site to the victim.

Discussion

Historical Overview

Historical outlook of this method reveals extensive literature. However, the methodology has remained largely unchanged since its improvement in the early 1960s. In 1861, Guy[9] got an idea that water along with some mud and other debris fragments channels to the various body organs when drowning takes place. While Brouardel[10] conducted series of experiment on dogs and found that a quantity of water equal to a third or quarter of the subject's blood volume enters the circulation when submersion takes place for about 20 minutes or more. But seventy years later Swann[11] reported that only after 3 minutes of submersion the original blood volume could be diluted by same volume of water. First discovery of diatoms in lungs was made by Hofmann[12] but a successful attempt was made by Revenstorf[13] who correlated this presence of diatoms in lung in solving the drowning mystery. This method was improved by digesting lung tissue with acid for the extraction of diatoms.[14] Detection of diatoms in blood and parenchymatous organs was successfully made by Incze[15]. This work was further carried out by Tamasaka[16] but this time source of diatoms was bone marrow. His studies helped to reach a conclusion that presence of diatoms in bone marrow indicates death by drowning but negative results of diatom studies carried out with this sample may therefore contradict drowning. A series of experiments on rats were carried out by Mueller and Gorgs[17] who reported that diatoms could easily be found in lungs but to a lesser extent in liver, kidney, brain and left side of the heart, and diatoms about the size of 30μ diameter could move into heart. Mueller[18] could not find any diatoms in the livers, kidneys and lungs of 30 non-drowned bodies and opined that diatoms can pass from air to other organs. Fluid from sinus of the basal bone was also only once recommended by Sveshnikov[19] in the history

of literature.[20] The fact that a great majority of diatoms are found in the digestate of peripheral tissue in cases of drowning, but that negative diatom findings do not rule out drowning was explained by Naeve.[21]

Contrary views about Diatom Test

There is a little controversy about the reliability of the diatom test; only a few workers have expressed contrary view about this method. Perhaps the most passionate condemnation of the diatom test is the fact that diatoms may be seen in the tissues and bone marrow of those who have died by means other than drowning and who are not found in water [22, 23, 24, 25, 26, 27, 28, 29, 30]. Some air born diatoms were also observed in lungs by [31], and when diatoms in the organs of still-births and premature births were discovered then it led them to conclude that the diatom test could not even be used on drowned infants. [32] reported their occasional presence in the lungs (rarely in the liver and kidney) of non-drowned bodies. Diatoms were found in the bone marrow of 15 out of 16 non-drowned subjects by [33]. In other medico legal observations on a dead body drawn up from the seabed [34] reported that large number of diatoms reached into the lungs by water pressure after death. Diatoms were found both in drowned and non-drowned cases by [30], and chances of recommending use of diatoms to prove death by drowning again faded. But it is suggested that if proper care is taken with all specifications and if the analysis is performed without contamination, it can serve as very significant supportive evidence in the investigation of the crime. This is due to diatoms that are ingested in other material or inhaled from the air [35, 36, 37]. As we know diatoms enter into the body through respiratory tract with the inspiration of water during breathing and it reaches to the lungs. This creates pressure and ruptures the alveolar walls of the lungs and makes the entry of diatoms into the circulatory system. From the circulatory system, these are circulated to various organs through blood. In the blood they are encountered by the defense system because firstly their size (average 25 microns) is more than that of the phagocytes or monocytes (15-20 microns) [38] and secondly because being covered by silica layer antibodies are not generated by the immune system against them. Presence of diatoms can be detected inside liver, kidneys, bone marrow and even in brain. The diatoms are not digested inside the body because of their siliceous exoskeleton. So, the presence of diatoms inside the body serves as corroborative forensic evidence. If present in domestic water, they have most likely been introduced residually from household cleansers. These are unique and recognizable species, used for their value as abrasives, and therefore should not confound the informed investigator [37, 39]. The presence or absence of diatoms is linked not only to their ability to navigate the alveolar-capillary interface through the circulatory system and eventually into the bone marrow, but is also highly dependent on size of diatoms (typically less than 30 microns) in order to embolize into the circulatory system and pass into the bone marrow [36] and the concentration of diatoms in bone marrow and other tissues which should be directly proportionate to the concentrations of diatoms in the water [40].This includes sampling of the suspected immersion fluid for comparison with the sampled tissue and bone marrow [41, 35]. Aspiration of water is required to transport the diatoms. The transportation process works precisely because the diatom's silica frustule is resistant to the mucus of the respiratory system and is able to embolize from the circulatory system into internal organs. Bone represents a closed system, requiring the circulation of blood to bring the diatoms into the marrow, where other organs such as the lungs may become invaded passively. Bone marrow is therefore preferable to all other sources of diatoms because those deposited are the ones most likely done so ante-mortem [36, 37] for this reason diatoms taken from external body cavities

or the lungs must be treated with caution [42]. It has been recommended, however, that diatoms from external body cavities (nose, throat, and ear canals) and those found in other tissues (lungs, brain, kidney, liver, and spleen) may provide additional positive clues to those found in bone as well as increase the proportion of positive test results, because larger diatoms may find their way into these places [35, 37]. However, this should only be used in instances when a body is not decomposed or the chest-cavity lies open because the chance of passive rinse greatly increases [43, 35]. Therefore diagnosis should be made using only closed organ samples, preferably bone marrow.

As previously mentioned, in instances where the body is open or the organs spoiled, or in the case of passive inhalation to the lungs, an assumptive diagnosis of drowning can be dangerous. The lungs or exposed organs may result in a positive test result while the bone marrow or closed kidney, spleen, or liver tissue may be negative [35]. While performing this test in forensic laboratory, risk factor of contamination also increases because centrifuge process requires a great deal of fresh water, which sometimes is not free of foreign diatoms [38]. The primary sources of this contamination are the reagents and the band saw or other tools used to collect or cut open the bone and other samples [42, 39]. However, contamination will not go unnoticed for long and to ensure an accurate comparison the site of a suspected drowning (putative drowning medium) should be sampled and tested for diatoms as well, and the results should be compared to those from the test bone marrow or other tissue [38]. This will also help to control for false positives resulting from inhaling airborne diatoms. A discussion of this issue explained the new modalities, suggesting that the diatom specialist is an advantageous partner to the medico legal investigation [5]. In numerous quality control tests of the procedure [39] found that if there are no diatoms in the water body of the suspected drowning, there are no diatoms present in the body tissues because the method of collection may not extract all the diatoms present in a sample and may miss diatoms altogether if only one or two individuals are present in the bone marrow, victims with heart conditions or other weaknesses can experience a laryngeal spasm, and there is a relatively low volume of blood circulating to and through the bone and bone marrow. Therefore investigation of closed organs becomes more important but concept of proper care must be kept in mind [35, 39, 42].

Supportive studies

Diatoms were also detected in marrow of sternum of the drowned subject by [44] and reported that this sample is more advantageous to use in comparison to the marrow of long bones because acid digestion time taken by this sample was less. In 1972 [45] reviewed previously used chemical methods employed to the investigation of drowning and reported that presence of a single diatom in femoral bone marrow is consistent with drowning as a cause of death. But this extreme view was no longer held, when [46] reported that the presence of a single diatom in femoral bone marrow would not now be the basis of such a diagnosis and suggested that results can usually be considered decisive but added that there will occasionally be doubtful results which will then have to be interpreted with the necessary cautions. Occurrence of diatoms and methods of their treatment have been explained along with a concluding remark that presence of diatoms in bone marrow is indicative of death by drowning and type of diatoms in bone marrow can also provide information about the type of water where death had occurred i.e. fresh or salt water[3]. In 1980, [20] critically reviewed role of diatoms in drowning cases. The arguments for and against the method were described, and the possible reasons for divergent opinion were explored. Important papers in Hungarian, Russian and French were translated and their essence was summed up. More suggestions were given by [41] that diatom test would be of much importance in the diagnosis of

drowning cases, origin of diatoms found in bone marrow is known i.e. matching of diatoms from both putative water medium and tissue of drowned body is must required for the success of this test. Some previous important reviews on diatoms and drowning have been published by [46, 47, 32, 45, 20]. Among the various works [2, 4, 5, 48, 49, 50, 51, 52] have made good efforts to make this study live and hopeful one. A review paper acknowledging some drowning associated diatoms was also published by [53].

Appreciable work

Technique provided by [54] was used by [40] to detect diatoms in the marrow of the sternum. In this technique the sternum was nailed on a board having inner surface upwards. Longitudinal strip of periosteum 10 cm x 3 cm was removed. Lid of bone was removed and marrow was collected with a curette. It was then put into a Kjeldahl flask and digested by 50 cc nitric acid within less than half an hour. The yellow transparent material so obtained was allowed to cool down and centrifuged. The rarely visible deposit was then poured on to a microscopic slide and examined under a cover glass. The magnification of 600 times was used. By the use of sternum further mutilation of body was avoided. Another advantage was the saving of time as compared to other methods and also the more quantity of diatoms recovered. He mentioned that the success of investigation depends upon the quantity of diatoms present in water. It was concluded that marrow obtained from sternum is advantageous than marrow obtained from long bones in detection of diatoms. Significance of diatoms was more enlightened by [55]. A yacht disappeared in English Channel in January and a body was found six week later on Belgian coast. It was transferred to England and was identified by fingerprints as one of the men who sailed on that yacht. Study of diatoms showed numerous diatoms in lungs, liver and bone marrow. The diatoms were found to be similar to those present off the Kent coast where the missing yacht was suspected to have abandoned. It proved the significance of diatoms. Work described by [55, 40] was further extended by [56] for the detection of diatoms in marrow of sternum. In the first method small pieces of lung tissue were removed from peripheral parts and were placed in round bottom Pyrex flask and digested by 50 cc nitric acid while heating the flask. The clear yellow solution so obtained was centrifuged. The deposit was poured on a microscopic slide and examined. In the second method, the sternum was sawn longitudinally and bone marrow was scooped out by means of a curette and digested in the same way. A clear yellow solution was obtained with a fatty layer at the top. The fatty layer was discarded and the deposit was examined for diatoms. Twenty drowned bodies were examined for diatoms. Diatoms were found in lungs in all cases and in sternum marrow in 12 cases. The control cases did not show any diatom and different types of diatoms were recovered. A paper on the diagnosis of death from drowning with particular reference to diatom method was presented by [57]. In this particular study, they took references from the earlier studies made by [13, 17, 18]. They studied the positive and negative aspects of diatom method. At last they concluded that the objections made were not so serious if proper care had been taken and the method is very useful in diagnosing death from drowning. Significance of diatoms in diagnosis of death by drowning was explained by [58]. They examined human lungs, livers, kidneys and brain of 43 cadavers removed from the natural water pools and bath tubs of Monroe County, New York from June 1963 to September 1966 and the tissue of 15 control cadavers with causes of death other than downing. For the animal experiments, six dead dogs were immersed in tank containing diatomaceous canal water. A conclusion was drawn that presence of diatoms in organs of systemic circulation is a good evidence that death occurred by drowning, but absence of diatoms does not

rule out the diagnosis of drowning and in areas where there is a great degree of air and water pollution with diatoms, the method may not be reliable. The available literature with particular reference to change of serum sodium, chloride, potassium and other chemical substances in cases of drowning was reviewed by [59]. It was stated that the variability of these and other abnormal chemical findings were dependent on a multiplicity of factors which were discussed, and made the scientific evaluation of the chemical changes difficult and limits their value as a diagnostic tool. Thirty four samples of sternum bone marrow were examined by [60], 31 of which were from subjects who had died from causes other than drowning. Of the remaining 3 subjects, one died from heart attack in the bath, and 2 from drowning. Results showed no diatoms in 33 sternum bone marrow samples, including the 2 samples from drowned subjects. Only one showed diatoms and it was concluded that it might be due to contaminated nitric acid. In his paper [3] reported that diatoms could be used as forensic evidence because of their use in the preparation of safe ballasts, paints and polishes etc. Occurrence of diatoms and methods of their treatment was also explained in his article. He reported that presence of diatoms in bone marrow is indicative of death by drowning and type of diatoms in bone marrow can also provide information about the type of water where death had occurred i.e. fresh or salt water and latter on [20] presented a review paper entitled "Diatoms and drowning" where he described the development of diatom method. Analysis of blood samples (left heart side) of 19 drowned bodies and 14 cases of natural death for the presence of diatoms was made by [29]. The drowning cases were autopsied 2 to 30 days after drowning, but the time had no effect on the number of macrophages or sand grains. Diatoms were found only in 3 of 14 drowning cases, rather common finding was crystals, probably the sand grains and no diatom was found in any of the fourteen control (non-drowned) cases. In 1984 [61] evaluated the diatom test in death of seventy professional divers where history and mode of death were accurately monitored. Drowning cases were divided into three groups. Group A shallow water divers (10-15 meters), Group B including three cases died of drowning in the Northern sector of the North sea between November to February and Group C including 4 divers working at depth of 50-100 meters and 3 at 100-300 meters. In Group D cases were other than drowning. in bone marrow, diatoms were detected in 12 cases and in only 3 cases could the morphology be related convincingly to control water samples. Analysis of the data in survey cases supported the value of diatom test in diagnosis of drowning cases. Both practical and theoretical implications of 10 cases of drowning taken from office of the Medical Examiner of Metropolitan Dade County in Miami, Florida were performed by [62]. He mentioned that during the 5 years study, 2617 homicides occurred and of these 12 or 0.46% were homicide by drowning. Practical implication of this study was how to "diagnose" homicidal drowning and how to "defend" this diagnosis. A paper dealing with 42 cases of fatal drowning occurring in Varanasi (India) for their epidemiological and medico legal including forensic pathological aspects was published by [63]. This report revealed that it was 36.62% of all 106 cases of asphyxial deaths and 4.75% of total 884 medico legal deaths autopsied among Hindu, Muslims, Christians and Unknowns of different age groups during the same period. Planktons were isolated from drowning lung tissue.

The use of electron microscopy for diagnosis of drowning cases was also suggested by some workers and some workers have also studied the ultra structural alterations in lungs of drowned bodies using Scanning Electron Microscope (SEM). Observations of the changes in alveolar walls in the rats caused by such asphyxia were presented by [64, 65]. But the use of SEM for identification and classification of diatoms from both water and post-mortem samples was made by [48] in London. They reported that this method does not alter the morphology of diatoms and makes

possible diagnosis of drowned bodies under specific conditions and moreover is simple, relatively rapid alternative with permanent photographic records suitable for taxonomic analysis. Again using light and electron microscopy significant quantitative difference between the number of diatoms in control and immersion cases in diagnosis of drowning was evaluated by [66]. Controversy about the penetration of diatoms in the alveolar-capillary barrier during drowning was addressed by [67] in Finland. Natural population of diatoms and an algal culture of *Phaeodactylum tricornutum* (PT) were investigated on adult male rats which were drowned and analyzed under SEM and Transmission Electron Microscope (TEM). This proved fact was a step forward in assessing the potentiality, reliability and limitations of diatom analysis on a new basis as a tool for the diagnosis of drowning.

For last 100 years, many scientists have employed various extraction methods individually and in combinations in order to isolate diatoms from water and tissues samples successfully. Among these approaches some of them were found to be superior to others. Nitric acid digestion is a worldwide known method for the extraction of diatoms. Apart from this some other extraction methods like Ultrasonic radiation, enzymatic digestion and physical methods such as simple centrifugation and gradient centrifugation are also employed for this purpose. Acid digestion method had been a revolution in the history of diatoms extraction and accepted worldwide. It is characterized by its low cost, easy to perform and gives excellent results. A review paper on the extraction methods of diatoms has been published by [68]. They have detailed various methods to extract diatoms from water and tissue samples. A devised method was suggested by [69], where lung of drowned body was dissolved by using a strong base or digestive enzyme. A new method was employed for destruction of diatoms in tissue samples [70]. Use of Soluene-350 (Packard Instruments), NCS (Amrsham/Searle Corp.) and Protosol (New England Nuclear), along with ultrasonic irradiation in place of acids were used. The tissues used were the liver and lungs of rabbits. They observed slight destruction of diatoms due to the ultrasonic irradiation but there was not so much difficulty in recognizing diatoms microscopically owing to their clear survival. Moreover this method was considered more advantageous over the method proposed by [71], where ultrasonic irradiation was not used. Use of Soluene-350 was further made by [72] for the destruction of tissue and detection of diatoms in drowning cases in fresh and sea water. It was observed that frustules of sea water diatoms were soluble in Soluene-350 but of fresh water diatoms did not and stated that this method is effective in diagnosis of drowning cases in fresh water. In 1987 [73, 74] presented two papers describing the detection of diatoms in blood and other tissue materials. This Japanese team invented a new method called 'Membrane filtering' (47 mm in diameter and 5 μm in pore size) to detect the diatoms through this membrane filter. Blood and tissue samples (liver, lung and kidney) of 39 cadavers suspected to be drowning were analyzed. In 32 inquest cases, two or more diatoms could be detected in 29 blood samples and one or no diatom was found in remaining 3 cases. Diatoms in the tissue and blood could be found clearly on the membrane filter, especially in tissue samples, some inorganic crystals and impurities appeared inevitably, but they did not interfere with microscopic observations. In another paper only blood samples of 17 cadavers were analyzed with this method. Of 16 cases 5-9 diatoms were detected in 5 cases, 10 or more and remaining 1 case did not produce any result. This method was helpful in solving the problems related to diatom test such as destruction, loss of diatoms and appearances of inorganic crystals during previously used method. In order to avoid the destruction of diatoms from acid and rigorous centrifugation treatment a new improved method of membrane filtering was also devised by [75] in 1975. Value of diatoms was considered significant and as a diagnostic tool [76]. The review of literature along with

practical work on the samples from 45 cases in British Columbia was taken into account which formed the basis of their research. They concluded that diatom findings in the tissues of drowned victims in diatom rich water can be reliable indicative of the drowning. Isolation and identification of diatoms and their comparison with the controls taken from the site, with knowledge of species identification along with geographical distributions, interpretations were made and a conclusion was drawn in favor of the diatom test. According to [42] the best known method used to diagnose drowning is the qualitative diatom analysis. His studies revealed that various species of diatoms can be grouped on the basis of environment conditions and diatoms analysis is a reliable supportive method for diagnosing death by drowning at least under Finnish conditions. Examination was conducted for the application of diatom test on different organs of individuals who died of drowning [77]. Diatom load in different organs was found to be positively related with breath/diameter of the frustule. Lungs, heart and kidney contained larger diatoms and smaller diatoms up to 10 µm were found in the brain and bone marrow samples. But another significant finding was the recovery of diatoms from the lungs, heart and kidneys of corpses of individuals in whom the cause of death was not drowning and brain and bone marrow were considered reliable samples for executing the diatom test. In a more elaborated and outstanding work in France, [35, 78] reported that results of river monitoring and profile of dominant taxa may be of immense help in those suspected drowning cases where reference water sample is not available they further added that 20 diatoms per 100 µL of pellet (obtained from 10 g of lung samples) and 5 complete valves for other organs can prove a positive diagnosis of drowning by diatom test.

Pollanen's work was a great achievement to prove the utility and validity of diatoms in forensic investigation of drowning cases in Toronto, Canada. Right from 1996 to 2000 his work has remained a big advantage in this field. In 1996 [2] threw some light on the use of diatom test and the necessary precaution that should be taken while performing this test in the laboratory because only small size diatoms (<30 µm) could be found in human tissue and presence of large diatoms is usually indicative of laboratory contamination rather than true drowning. He further added that presence of diatom in the bone marrow gives an indication that person was breathing at the time of drowning. Diagnostic value of the diatom test after doing a retrospective analysis of 771 cases of drowning in Ontario (Canada) was accessed by [39]. The cases were analyzed at the office of chief coroner for Ontario from 1977-1993. Bone marrow from intact femur or rarely an intact kidney was used for the extraction of diatoms. Just after this work [36] again studied 52 cases of fresh water drowning with diatoms in femoral bone marrow and sample of putative drowning medium were also collected. The frequency of positive test was determined and correlated with season of drowning. 90% of the cases gave positive results and there were great seasonal variations in frequency of the diatom test in accordance with diatoms in bone marrow and putative drowning. In 1998 [37] again studied 6 cases of homicidal drowning in which diatoms played useful role. In a case [79] demonstrated two important points in the assessment of diatom evidence in cases of suspected drowning. Diatoms of the *Chaetoceraceae, corethronaceae, Bacteriastraceae, Rhizospleniaceae* and *Leptocylindraceae* species were found to be easily destroyed by acid digestion method because they are lightly silicized. Absence of such species after acid treatment is therefore to be expected and must not be interpreted as 'diatom absent'. After examining 133 cases of 'typical' and 'atypical' drowning and samples of putative drowning mediums [43] reported that diatoms were present in 70 samples of water, and in 11 samples they were absent. Out of 70 cases with diatoms found in water, in 51 (73%) diatoms were found neither in tissue nor in blood, while 19 cases diatoms were found. In these cases variety of diatoms found in water were always similar to

those found in biological samples. A new instrument "Can" for the destruction of tissue material for forensic analysis of diatoms and of metallic poisons was developed by [80] in China. Time saving, no contamination, simple in operation, safe and reliability were its outstanding features. Disarticulated limbs of 5 drowned bodies were undertaken for analysis of the presence of diatoms by [5]. They considered diatom test as an adjunctive investigation and bones like femur and tibia were also assessed using anthropological methods to give estimation of the sex, age, race and stature of the drowned individual. Emphasis was made on the detail study of destiny (quantity), species (quality) and description of valve (morphology) of diatom for every sample before the diagnosis of death by drowning [50]. It was reported that a specific density and size of diatoms in water helps to reach them into the body organs from any putative drowning medium. After conducting tests on 22 human cases suspected for drowning and one human case of death other than drowning in Macedonia [81] concluded that diatom method was represented as one of the most valid methods for the diagnosis of drowning but if the analysis is properly conducted. Forensic palynology with special reference to diatom and its evidentiary value in forensic investigation was detailed by [82]. An interesting article has been published by [83] where he explained the role of diatoms in solving suspected drowning cases and difference in their diversity from one water body to another. Enumeration of two case studies emphasizing about the medico legal application of both maggots and diatoms simultaneously in the cases with advance decomposition was conducted and published by [84]. They opined that this simultaneous analysis may reveal fairly sound conclusions relating to postmortem intervals and drowning mode of death especially in decomposed cases. The most frequent features of death by drowning as a basis for differential determination of manner of death of dead bodies recovered from water and the usefulness of diatom test for the same purpose was beautifully enlightened by [51]. In another attempt [52] presented modern analog techniques suggesting that lungs and clothing samples have statically significant similarities to control samples from shallow water habitats. In these quantitative reconstruction techniques, a correlation was made between diatom samples from study area and diatoms recovered from clothing and lungs in order to support drowning a cause of death and localization of site of drowning. For diatom nanotechnologists a literature guide was presented by [85]. This paper includes book references, courses, culture collections, journals, libraries, review articles, societies and meetings and web pages of general interest. A review of reported diagnostic methods is discussed in order to provide guidelines, which can be used in current forensic practice has been elaborated by [86]. Some important aspect of the difficulty in diagnosis of drowning cases has been described here and it is emphasized that the ideal diagnostic test as definite proof for drowning still needs to be established. A review paper based on of past and contemporary science and application of diatom test in an attempt to renew general interest in this significant test was forwarded [5].

Role of diatoms in localization of drowning site

Another important aspect regarding diatoms in forensic investigation is to locate the site of drowning on the basis of identification and comparison between diatoms from post-mortem and water samples from where drowned body was recovered. Ecological and morphological studies have been undertaken by various botanists but in the field of forensic science some workers have also made attempts in this purpose. None of the workers in this field have been diatomologists, with the exception of [31, 87], and they have provided some knowledge about the diatoms. Some previous studies have advocated this aspect of determination of drowning site on the basis of

diatomological studies. An analysis of the water samples of various ponds and lakes of Delhi was undertaken [88]. Two population maxima one in spring and another in autumn and 14 species of diatoms were observed. This data-base was made to solve suspected drowning cases by making a link between drowned body and actual site of drowning. In Finland a six cases study [42] helped to reach a conclusion that even general aquatic environmental conditions like depth of water, pH of water, type of water whether salt or fresh could be traced by observing these diatom species. Further addition to this aspect was made by [2], and matching of diatoms from both water and tissue samples was highlighted to identify the place of drowning. Application of molecular biology to the diagnosis of drowning through PCR was also appreciated in the evolution of the diatom test. A detailed analysis of 771 cases of drowning helped [39] to reach a conclusion that diatom test can identify approximately one in three victims of freshwater drowning, and may be useful in assessment of deaths occurring in bathtubs and in selected circumstances when drowning medium is not available and body is not decomposed. In the following paper [36] after analysis of diatoms in putative drowning mediums, concluded that (i) winter months have the highest frequency of samples devoid of diatoms (ii) small pinnate frustules are most commonly associated with drowning, particularly in non winter months (iii) these diatoms are representative of site of drowning and (iv) in 10% cases where diatoms did not match in both samples due to remote geographical origin of these diatom from the site of drowning. In another report [37] revealed some information regarding regional and temporal variation in the type of diatoms in the same body of water, on this occasion; extensive sampling of water at the suspected site of drowning at different times and water depth was suggested.

A more elaborated and outstanding work to associate drowned body with particular aquatic body was done by [35] in France. They conducted a continuous river monitoring programme of diatoms in the diagnosis of drowning cases where drowning site was under suspicion or not available. Their attempt became successful when in year [78] compared the microflora of previously made data base with microflora recovered from the tissue of drowned bodies to locate the actual drowning sites. A concept made by [50] emphasized on the detail study of density (quantity), species (quality) and description of valve (morphology) of diatom of every sample before diagnosis of death by drowning cases because these characteristics play an important role for the diatoms to reach into the body organs of a drowned person. On the basis of observation taken from 22 human and two series of rat tests by [81] it was reported that diatom test represents one of the most valid for detecting the cause, place and time of drowning. Another support in this study was provided by [89]. This study recorded distribution of diatoms in Yoronjima and took it as a model study to evaluate the application of diatom testing for the diagnosis of death by drowning. The numbers of diatom were observed varying owing to the difference of location, the distance from shore and the depth of sea and the tide. These results suggested that a careful analysis of the putative drowning medium is essential for an accurate diatom test for drowning off islands in the open sea.

Conclusion

Diatoms found inside the body of a drowned victim may serve as corroborative evidence in the diagnosis of cause of death. It can be ascertained that whether the death is ante-mortem or post-mortem. Diatoms are not always there in all of the drowning cases but if present and present in distant organs in abundance they definitely provide a positive evidence in favour of ante-mortem drowning. There is a lot of controversy about the reliability of diatom tests. Many authors do not consider this as a valuable and a fool proof method. The fact which supports their opinion is that

diatoms are not only inhaled through water, they can also be inhaled through air as they can also be found in the air and from there they can gain entry by the respiratory system. But the studies made by [40, 37, 39, 42] form a view that diatom test is very reliable in ascertaining ante-mortem or post-mortem drowning by taking each and every aspect with great care and keen observation. A definite conclusion can be drawn if proper care is taken to avoid every sort of contamination and by knowing all necessary specification of the diatom test, it can provide a great assistance in the investigation of drowning cases. According to this criterion, the diatoms found in the blood and organs of the victim (such as femur), must be the same. This is to refute the objections of many pathologists who assert that diatoms are ubiquitous in human tissues. Researchers have found diatom like particles in hepatoportal circulation indicating thereby that they might have entered through ingested food or water. It is implied that they would then get distributed among the tissues of the body during the life of the person. The author of course doubts this assertion, reminding us that such a process would entail completion of a very hazardous journey by these tiny particles, which is not likely. They have to dodge Kupffer cells in the liver and phagocytes in the blood (which are live and active) in order to find a safe place in the terminal organs. Yet the application of the *Criterion of Concordance* would be sufficient to silence most critics in a court of law. A number of methods are described for the digestion of the tissue for the isolation of diatoms by doing minimum damage to the frustules and if we observe keenly we can say that membrane filtering method given by [73, 74] provides better results. The residue if seen under Scanning Electron Microscope probably gives the best view and best results. As earlier an assumption was made to use molecular biology methods for the development of this method [2], and now because of the easy availability of advance tools that method can be easily adopted. Possibility of providing DNA based species level identification enhances with this approach. It will switch the comparative study of conventional and putative PCR based methods towards an interesting turn. No doubt diatom test has been an excellent remark in the diagnosis of drowning cases but somehow it can also be used more potentially in those cases where recovered body is under suspicion for drowning site or where drowned body is found on land [35]. In examining the modality, however, it became clear that some of the current criticisms and unspoken shortcomings could be addressed with original thinking and new methodologies. A fresh outlook is necessary to use of this important application for medico legal investigations.

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