

A Systematic Review on Various Diatoms Species Associated with Drowning

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Review article

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Abstract

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Diatoms are microscopic unicellular photosynthetic organisms with widespread distribution. Diatoms come under a branch of phycology with membrane bound nucleus that separates them from prokaryotes. When the person gets drowned in water containing diatoms then due to force full aspirations they get enter into the lungs and from lungs they get lodged into the distant organs of the body. Various tests have been developed so far for the diagnosis of drowning deaths. Diatoms test have been evolved as one of the most important tests for determination of drowning deaths due to its higher specificity and sensitivity. The extraction methods of diatoms from tissue samples is based on digestive capability, reclaiming ratio of diatoms, destruction of diatoms samples and time consumed for complete diatom digestion. This paper reviewed the recent year's progress in diatom test, samples used and species of diatoms associated with drowning.

Keywords: Diatoms; Frustules; Drowning; Acid digestion; Microscope; Species

Introduction

Drowning is one of the most serious and neglected health problems. Throughout the world about 42 people died every hour and 3.7 lakh people died from drowning every year. In India out of total cases of un natural deaths 9.4% peoples were died due to drowning, which is second most common cause of death after road accident [1]. Drowning is third leading cause of unintentional death worldwide which accounts for 7% of all injury related deaths. Low- and middle-income countries accounts for about 90% of unintentional drowning deaths. Children, males and individuals with more access to water are most at the risk of drowning. About half of the drowning deaths occur in WHO Western Pacific Region and WHO South East Asia Region. There is uncertainty in the estimation of global drowning deaths. Official data categorization methods for drowning deaths exclude intentional drowning deaths (homicide or suicide) and drowning deaths caused by flood disasters and water transport incidents [2].

Death due to drowning is defined as a death due to submersion in liquid and mechanism of acute drowning is hypoxemia and irreversible cerebral anoxia [3]. Various signs of drowning are hair and clothing wet, washerwomen symptoms on palm and sole, white leathery froth detected in nostril, mouth and trachea. Lungs become voluminous and cover chest wall with impressions of ribs are found. Dissection of lungs shows white froth with blood oozes out with a sound of crackling [4]. When the body is recovered from water there is usually a suspicion that whether the cause of death is a drowning or body has been dumped in water body to simulate it to be a drowning. Diatom test has emerged as one of the most reliable tests to diagnose the cause of death in drowning cases. Diatoms are microscopic unicellular algae which can be used for the diagnosis of drowning deaths. Presence of diatoms in lungs and bone marrow is an indicator of death due to drowning [5]. In forensic science one of the main issues of diagnosis of drowning death in case of submerged corpses occurred when the corpse is heavily putrefied or decomposed. Diatom test is based on the recovery of diatoms from organs of drowned victims. In case of putrefied bodies diagnosis of drowning is one of the difficult tasks and diatom analysis can provide supplementary evidence [6].

Conventional methods used for diatoms test involved extraction of diatoms from tissues and water samples, membrane filtration or centrifugation for the collection of diatoms and identification of diatoms on the basis of morphological features by using light microscopy or Scanning Electron microscope [7]. Diatoms have a siliceous cell wall with distinctive shapes and ornamentations. The ornamentation found on the silica frustules of diatoms is species specific and thus provide important information about their shape and sizes which can be used for their identification and classification. On the basis of shapes diatoms are classified into two specific groups one is centric with radial symmetry and other are pinnate which exhibits bilateral symmetry.

Methods for Literature Search Strategy

Literature search strategy for the papers related to diatoms species associated with drowning deaths was searched on Google research scholar, research gate and Clarivate Analytics Web of Science. From these twenty papers have been identified pertaining to diatoms species associated with drowning deaths. Additional papers were also retrieved from the retrieved list of reference papers. Then the data obtained from the research papers was put on the master charts to get the relevant information and then analyzed after review of literature.

Discussion

Recent years various methods have been developed for diagnosis of diatoms from tissue samples by enzymatic digestion methods by using proteinase kinase K [8], microwave digestion-vacuum filtration-automated scanning electron microscopy (MD-VF-Auto SEM) [9,10], Molecular biological techniques [11-14] and loop mediated isothermal amplification (LAMP) [15]. From all these methods acid digestion method is still widely used for the detection of drowning deaths (Table-1).

Diatom test can not only determine the mode of death as drowning but it can also determine the site where the drowning has taken place. Various researches conducted throughout the world showed that some of the species of diatoms are site specific and thus they can be considered as endemic. Diatoms also show variations with temperature, pH and season. Hence diatomological mapping is of keen interest by the forensic pathologist in the near future [16].

Diatomological mapping helps in generation of baseline data which aids in forensic investigation for diagnosis of drowning deaths, in location of diatoms diversity in particular location and in environmental science and Botany in assessment of water quality in particular region. Cohelo et al in their 37 cases of drowning deaths showed that there is a strong relationship between the diatoms detected in control water sample recovered from the drowning site and tissue samples of drowned victims [17].

Continuous monitoring of water bodies for the presence of diatoms is very essential for the detection of diatoms. Sometimes body of a drowned victim get drifted to other sites due to water currents and thus if diatom database is available then we can locate the exact site where actual drowning has taken place. In recent years some of diatomological mapping by studying distribution and characterizations of diatoms in various water bodies of China was done by Bi et al [18], Ren et al [19], Du et al [20], Bai et al [21] and Tian et al [22]. In India diatomological mapping in some of water bodies were done by Thakar and Singh [23], Vinayak, et al. [24], Saini, et al. [25], Sane, et al. [26], Rana and Bhandari [27] and Mishra and Kumar [28]. Suphan and Peerapornpisal recorded two hundred and fifty three species of benthic diatoms in Meckong river in Thailand [29].

Various factors are responsible for the distribution of diatoms in body organs. These factors depend on the density of diatoms in drowning medium, filtration of diatoms into the body when they passed from drowning medium to blood stream and size of diatoms that percolates into the body [30].

Various biological and thanoto-chemical tests like Haemodilution tests [31], sinus fluid [32], microorganisms like algae [33], paranasal sinuses [34], Chemical constituents like strontium [35], diatom test (Table 1) and various markers like magnesium, sodium, chloride, calcium [36] are utilized for the diagnosis of drowning deaths. From all the methods utilized for drowning deaths, diatom test is still considered as "golden standard" [37].

Complete destruction of tissue samples is required for the extraction of diatoms from tissue samples. Various studies conducted throughout the world showed that different internal organs can be used for qualitative and quantitative analysis of diatom test. If the diatoms are detected in the organ samples then cause of death as a drowning can be supported. Various studies conducted on diatom test showed that the lungs sample is commonest organ used for diagnosis of drowning deaths [38,39]. Besides lungs other tissue samples used for diatom test are Left heart blood [40], Sternum, femur, tibia, lungs, spleen, kidney, heart, clavicle, Peritoneal fluid, Pleural Cavity fluid, Liver, Sphenoid fluid, brain and stomach contents (Table 1). Bone marrow tissue sample is considered as the most appropriate sample for diatom test as it is least effected to contamination and putrefaction.

Various studies conducted on diatom test showed that the pennate diatoms were detected more in number in the tissue samples of drowned victims as compared to the centric diatoms. This might be explained on the basis of the shape as pennate diatoms are most penetrative as compared to centric diatoms. Moreover, the literature cited also showed that the pennate diatoms are more abundant in the control water samples as compared to centric diatoms (Table 1).

The diatom test has been extensively used by forensic laboratories for the diagnosis of ante mortem or postmortem drowning in biological samples and their comparison with the putative site of drowning. If the diatoms detected in tissue samples matches with the control water sample then it can act as corroborative evidence that death due to drowning has occurred in same water medium [41].

The identification of the diatom taxa in terms of species and dominant species comparison between the sample tissues and control water samples is required to detect drowning deaths by forensic pathologist. The diatom valves or fragments recovered from the tissue and control water sample must be analyzed at lowest possible level up to species or variety. They must be visually documented in terms of photographs or digital prints. Two similarity indices are used in research process i.e. Species Index (SI) and Dominance Identity (DI). SI is used to compare the number of taxa between two samples and can be calculated as:

$$SI_{1,2} = S_{1 \cap 2} / S_{1+2} * 100[\%]$$

 $S_{1\cap 2}$ is the number of species of diatoms species common in communities of samples 1 & 2 and S_{1+2} is the total number of diatoms in two diatoms communities in samples 1 & 2.

Dominance identity between two samples can be calculated by DI as

$$DI_{1,2} = \Sigma q_i [\%]$$

 $DI_{1,2}$ is the similarity between samples 1 & 2 and Σq_i is the smaller two relative abundances of species i. Both the indices are calculated in terms of percentage and varied from 0% (similarity none) to 100% (similarity matches completely) [30,42].

Name of Author	Tissue samples used for study	Methods used	No. of sample (s)	Percentage of cases positive or negative	Diatoms species detected
Pal, et al. [43]	Sternum, femur, tibia, lungs, spleen, kidney and heart	Acid Digestion Method	N=66	Positive=62 (93.93%) Negative=4 (6.06%)	Navicula, Nitzschia, Asterionella, Cymbella, Pinnularia, Coconesis, Diatoma, Cyclotella, Gyrosigma, Diplonesis, Aulacoseira, Stauronesis, Coconesis, Tabuleria, Epithemia, Melosira

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	Sternum, Femur,				
Kaushik, et al. [44]	Clavicle, Peritoneal Fluid, Pleural Cavity fluid, Tibia, Humerus, Liver, Spleen, Kidney	Acid Digestion Method	N=17	Positive=12 (70.58%) Negative=5 (29.41%)	Cymbella, Cyclotella, Diatoma, Epithemia, Aulacoseira, Gyrosigma, Coconesis, Diadesmus, Tabellaria, Amphora, Synedra, Rhoicosphenia
Gunatilake & Gooneratne [45]	Lungs, Kidney, Bone marrow	Acid Digestion Method	N=35		Cyclotella, Navicula, Nitzschia, Frustulia, Gomphonema, Pinnularia, Cymbella, Cocconeis
Magrey & Raj [46]	Sternum, clavicle, Lungs and Femur	Acid Digestion Method	N=31	Positive=09 (29.03%) Negative=22(70.96 %)	Navicula lanceolata, Navicula oblonga, Gomphonema gracile, Nitzschia gracilis, Cymbella cymbiformis, Gomphonema spheroporum, Nitzschia frustulum, Cymbella ventricosa, Cocconesis placentula, Amphora, Amphipleura, Aulacoseria, Meloseira, Hantzshia, Eunotia Epithemia
Kumar, et al. [47]	Sternum, clavicle and Femur	Acid Digestion Method	N=7	Positive=04 (57.14%) Negative=3 (42.85%)	Coscinodiscus centrales, Navicula cuspidata, Pinnularia viridis, Cymbella tumida,
Malik, et al. [48]	Sternum, clavicle, lungs and Femur	Acid Digestion Method	N=4	Positive=03 (75%) Negative=1 (25%)	Navicula lanceolata, Navicula oblonga, Gomphonema gracile, Nitzschia gracilis, Cymbella, Navicula radiosa, Cymbella cymbiformis, Gomhonema spheroporum, Nitzschia frustulum, Cymbella ventricosa, Cocconeis placentula
Vinayak, et al. [49]	Femur, blood from left side of heart	Acid Digestion Method	N=2	Positive=1 (50%) Negative=1 (50%)	Navicula, Asterionella, Cocconeis, Caloneis
Zhao, et al. [9]	Liver, kidney, bone marrow	Microwave digestion- vacuum filtration- automated scanning electron microscop y method	N=2	Positive=1 (50%) Negative=1 (50%)	Actinocyclus normanii, Amphora pedicultus, Aulacoseira ambigua, Aulacoseira crassipunctata, Cocconeis placentula var. euglypta, Cyclotella meneghiniana, Cymbella affinis, Cymbella delicatula, Cymbella laptoceros, Cymbella tumida, Fragilaria capucina, Hantzschia amphioxys, Gomphonema clavatum, Gomphonema parvulum,Gomphosphenia lingulatiformis, Lemnicula

					hugarica, Luticola nivalis, Luticola muticopsis, Stauroneis anceps, Tabularia fasiculata, Tabularia tabulate, Navicula radiosa, Navicula symmetrica, Nitzshia clausii
Chun-Yen Lin, et al. [50]	Sphenoid sinus fluid and lung tissue	Acid digestion method	N=120	Positive=83 (69.16%) Negative= 37 (30.83%)	Achnanthes, Amphora, Aneumastus, Bascillaria, Brachysira, Caloneis, Chaetoceros, Cocconeis, Coscinodiscus, Cyclotella, Cymatopleura, Cymbella, Denticula, Diatoma, Diploneis, Entomoneis, Epithemia, Eunotia, Fragilaria, Frustulia, Gomphonema, Gyrosigma, Hantzschia, Hemidiscus, Licmophora, Meloseira, Navicula, Neidium, Nitzshia, Pinnularia, Planothidium, Pleurosigma, Rhaphoneis, Rhoicosphenia, Skeletonema, Stauroneis, Staurophora, Stephanodiscus, Surirella, Synedra, Thalassionema, Thallassiosira
Caniero, et al. [39]	Left Lung fragment	Acid digestion method	N= 1	Positive=1 (100 %)	Aulacoseira granulate, Actinocyclus normanni, Pleurosira laevis, Cocconeis placentula, Fragilaria, Fragilariaforma, Eunotia, Encyonema, Gomphonema, Achnanthes, Pinnularia, Frustulia, Navicula, Nitzschia
Bąk, et al. [51]	lower lobe of left lung (150 g), the entire kidney (145g), 200 g of liver and a 10 cm long fragment of the rib approximately 3 g of marrow).	Acid digestion method	N= 1	Positive=1 (100 %)	Actinocyclus normanii, Aulacoseira ambigua, ulacoseira, Cyclostephanos dubius, Cyclostephanos invisitatus ,Cyclotella comta, Cyclotella meneghiniana ,Cyclotella meneghiniana,Cyclotella ocellata, Cyclotella radiosa, Cyclotella, Melosira,Paralia sulcata,Stephanodiscus hantzschii,Stephanodiscus neoastraea, Stephanodiscus parvus,Thalassiosira visurgis, Thalassiosira,Achnanthes, Achnanthidium minutissimum, Adlafia suchlandtii, Amphipleura pellucid, Amphora copulate,Amphora

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						placentula var euglypta, Melosira

					varians, Hantzschia amphioxy, Nitzschia palea, Navicula
Kakizaki [52]	lower lobe of the right lung, lower lobe of the left lung, right kidney and the left lobe of the liver	Acid digestion method	N=1	Positive=1 (100 %)	accomoda Achnanthes, Achnanthidium, Aphora, Asterionella, Cocconeis Cymbella, Encyonema, Fragilaria., Frustulia, Gomphonema, Navicula, Nitzschia, Pinnularia, Planothidium,Cymatopleura, Surirella, Ulnaria ,Aulacoseira, Cyclotella, Melosira varians, Diploneis elliptica, Actinoptychus, Thalassiosira, Coscinodiscus, Bacteriastrum , Chaetoceros,Biddulphia, Odontella,Diploneis(panduriform), Fallacia,Pleurosigma, Grammatophora, Plagiogramma,Rhaphoneis,Rhopal odia, Thalassionema,Trachyneis,Navicul a schonkenii,Nitzschia panduriformis
Yoshimura, et al. [53]	Lung, liver and kidney tissue	Soluene 350	N=2	Positive=2 (100 %)	Meloseira, Staurastrum, Navicula, Cymbella and one species of Zygnemataceae
Muccino [54]	brain with meninx, lung, liver, kidney and femoral bone marrow	Acid digestion method	N=139	Positive=82 (59%) Negative=57 (41%)	Cyclotella,Cymbella prostate, Cymbella vetricosa Cymbella lanceolata,Cymbella tumida, Diatoma elongatum, Dimatoma vulgaris,Ghomphonema sp., Ghomphonema olivacea,Navicula lanceolata,Navicula viridula, Navicula sp.,Caloneis amphisbaena, Caloneis ventricosa, Cocconeis pediculus,Cocconeis placentula, Cocconeis sp., Fragilaria crotonensis Fragilaria virescens, Filiform Melosira sp.
Ludes et al. [55]	Lung, Brain, Kidney, Liver	Chemical digestion method, Ashing method, Enzymatic digestion method	N=12	Positive=12 (100%)	Navicula, Frustulia, Cyclotella, Nitzschia, Melosira, Fragilaria, Gomphonema, Synedra, Cymbella, Tabellaria
Kakizaki, and Nobuhiro [56]	Liver, Kidney and Lungs	Qiagen Proteinase K, Qiagen Buffer ATL	N=10	Positive=10 (100%)	Achnanthidium, Amphora, Asterionella, Cymbella, Diatoma, Eunotia, Fragilaria, Gomphonema, Navicula, Nitzschia, Pinnularia,

		and 5N hydrochlor ic acid			Surirella, Ulnaria, Cyclotella, Melosira, Actino-ptychus, Bacteriastrum, Biddulphia, Chaetoceros, Climacosphenia, Diploneis (panduriform), Grammatophora, Licmophora, Rhizosolenia, Campyloneis, Rhaphoneis, Thalassionema
Ago, et al. [57]	Upper and lower lobes of left right lungs, spleen, left and right kidneys, liver	Acid digestion method	N=10	Positive=9 (90%) Negative=1 (10%)	Navicula, Nitzschia, Melosira, Fragilaria, Pinnularia, Synedra, Cocconeis, Cymbella, Cyclotella
Coelho, et al. [17]	Lung, kidney, liver, bone marrow, stomach contents	Acid digestion method	N=37	Positive= 63%	Fragilaria fasciculata, Cyclostephanus dubius, Fragilaria crotonensis, Cyclotella species, Achnanthes lanceolata, Cocconeis neothumensis, Cyclostephanus dubius, Cyclotella meneghiniana, Aulocoseira distans, Cocconeis placentula, Achnanthes minutissima, Fragilaria elliptica, Eunotia minor
Pachar and Cameron [58]	Lung tissue, Kidney, liver, brain, bone marrow	Acid digestion method	N=40	Lungs positive = 93.7%	Epithemia, Nitzchia, Cymbella, Actinoptycus, Raphoneis, Synedra, Achnantes, Cyclotella, Surrirella, Navicula, Gomphonema, Cocconeis, Melosira, Cocinodiscus, Plagiogramma, Cymatosira, Cyclostephanos

Table 1: Comparison of results of the studies conducted in different parts of the world.

Positivity and validity of diatom test is based on the number of diatoms valves recovered from the tissue samples and their comparison with the tissue samples. Pal, et al. [43] reported 93.93% positive cases in drowning deaths, Kaushik, et al. [44] in 70.58%, Magrey and Raj [46] in 29.03%, Kumar, et al. [47] in 57.14%, Malik, et al. [48] in 75%, Vinayak, et al. [49] and Zhao, et al. [9] reported in 50% of cases. Caniero, et al [39], Bak, et al [51], Benzamin, et al. [38], Kakizaki [52], Yoshimura, et al. [53], Ludes, et al. [55] and Kakizaki and Yukawa [56] reported positivity of diatoms in 100% of cases. The negativity of diatoms in drowning deaths might be due to low abundance of diatoms in drowning medium, destruction or loss of diatoms during processing or inappropriate organ samples used for the study. Various studies conducted on diatom test showed that number of valves recovered from the tissue samples is based on the type of tissue sample used for the diatom test. Therefore,

a standard had to be set up to draw the conclusion regarding positivity or negativity of diatom test.

One of the most criticisms for the validity of diatom test is detection of diatoms in the tissue samples of nondrowned victims. Buri, et al. in their study showed that detection of diatoms in tissue samples of non-drowned bodies might be due to abundance of diatoms frustules in non-vegetarian cooked food stuff, inhalation of air in a atmosphere containing material used for manufacturing the building like paper, cement and paint etc. and long term exposure of body in a water containing diatoms [59]. Disposable equipment's should be used to avoid the contamination between the samples, appropriate quantity of sample should be taken and quality of sample preparation should be high with minimum residue in the digestive sample [30]. Levkov, et al. in their study showed that rapid death in water medium due to shock or cardiac dysfunction at low temperature, due to diseases and use

of alcohol or drugs also causes the decrease in time period for the drowning deaths which may cause in negativity of diatom test [60]. Rana and Manhas in their study showed that rapid death due to drowning, less abundance of diatoms in putative site of drowning, inefficient methods of extraction and detection causes negative results in drowning related deaths [61]. Therefore, discrimination between the positive and negative results under such types of circumstances is one of the most challengeable tasks for the forensic experts.

The literature cited on the diatoms study showed that very less data is available regarding the size of the diatoms associated with drowning. The size of diatoms in natural habitat varied from 2µm-200 µm [62]. Hurlimann, et al. in their study showed that maximum length of diatom valve detected in bone marrow should be less than 40 µm. If the length of diatom is more than 40 µm this might be due to post mortem contamination or penetration from gastrointestinal wall [30]. Levkov, et al. in their study showed the minimum to maximum size of diatom valve in ten cases of drowning deaths varied from 9-122 μm in lungs, 7-117 μm in brain, 13-187 μm in liver, 8-85 µm in heart and 11-122 µm in kidney [60]. Similarly, the studies made by Krstic, et al. reported maximum length of diatom is 50 µm in tissue sample [63], Pachar and Cameron reported 30 µm [58] and Lunetta et al reported 110 µm [64] in their respective studies.

Sometimes due to certain environmental conditions diatoms may be scanty or absent in a particular season which causes negative results pertaining to drowning deaths. Therefore, to increase the sensitivity and specificity of the diatom test other phytoplankton besides diatoms can also be used for the diagnosis of drowning deaths [65]. Lunette and Modell in their study showed that presence of phytoplankton other than diatoms can be detected in the drowned bodies and found that aquatic organisms belonging to division chlorophyta, Dinoflagellates, invertebrates, protozoan ciliates in the blood of drowned victims [66]. Díaz-Palma et al standardized the methods for recovery of phytoplanktons from drowned victims and showed that dinoflagellates and some chlorophytes have cell walls and structures which are resistant similar to diatoms [67]. Yoshimura et al in their study showed that phytoplanktons can be extracted from tissue samples by using soluene-350. Diatoms and some species of green algae were detected in the tissue sample and control water sample by using this method [53].

Conclusion

Diatom test has emerged as an important test for forensic laboratories to diagnose the cause of death in drowning cases. Presence of diatoms in the tissue samples can act as supportive evidence that death has occurred due to drowning. If a person is already dead before entering into the water the diatoms could not be detected into the internal organs of the drowned victims. Detection and comparison of diatoms with tissue and control sample is required to rule out the positivity or negativity of diatom test. Validity of the diatom test is also based on the number of diatoms valves recovered from the tissue samples of drowned victims. Various methods have been developed so far for the diagnosis of drowning deaths but still diatom test is considered as a golden standard. Molecular biological techniques. ADIAC and phytoplankton mapping may aid up the diatom test in near future.

Ethical approval: Ethical guidelines were respected.

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