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Detection of diatoms in autopsy of normal population vis-à-vis drowning

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Abstract

Drowning being third leading cause of un-intentional injury death globally after road traffic accident & falls, its correct diagnosis is very important in legal proceedings. Although detection of diatoms in viscera has been employed as a reliable method for diagnosis of drowning, there is ongoing debate in scientific community over the validity & reliability of this test. So, it was thought proper to compare detection of diatoms in sternum and liver of 50 drowning and 50 non drowning cases. The diatoms were detected in sternum of 6% cases and in liver of 26% cases in non-drowning as compared to 44% positivity in sternum and 82% positivity in liver of drowning cases. So, it was concluded that accepting diatoms test as a gold standard test is a matter of debate.

Keywords: Drowning, Diatoms, Asphyxia, Algae, Bone marrow

Introduction

Drowning is a form of asphyxia death in which the atmospheric air is prevented from entering the lung by submersion of the body in water or any other fluid medium.[1] The vast majority (approximately 97%) of all drowning deaths occurred in low and–middle income countries. In India, in 2000, there were an estimated 86000 deaths due to drowning, which was the fourth leading cause of unintentional injury deaths and contributes to 17% of total global burden.[2] In the case where there are eyewitness accounts of accidental drowning or suicide, followed by autopsy and histopathological examination of the body recovered from water shortly after death, the medical Jurist may have little difficulty in establishing drowning as a major contributory factor causing death. But in cases where the body is recovered several weeks or months after death, the diagnosis of death by drowning is not very easy and far from straight forward.[3] Extensive research has been conducted over many years to identify a test which can be a gold standard for diagnosis of drowning deaths.

Guy (1861) observed that in drowned subjects i.e. who died due to inhalation of water, trachea and larger bronchial tube contain water which sometimes penetrates to the minutest ramification and occasionally carries with it portion of slime or mud and fragments of aquatic plants. Entering of water in blood circulation in case of submersion was described in a series of

experiments of drowning dogs of Brouardel and Vibert (1880) and later by Swann and Spafford (1951), who however found that the process was much faster than previously described. It fell to Revenstorf (1904), however to examine the penetration of diatoms into the lung of drowned subjects and it is this work which began the association between diatoms and the diagnosis of death by drowning[4].

Diatoms are major groups of algae, and are one of the most common types of phytoplankton. Most diatoms are unicellular, although they can exist as colonies. Diatoms are eukaryotes belonging to class bacillariophyceae. They have complex cell wall which is impregnated with silica (hydrated silicon dioxide) and contains yellowish brown chloroplast diatoms secretes hard siliceous outer skeleton called frustules. They are chemically inert and almost indestructible (although soluble in strong alkali) and tend to persist for long period of time. Frustules are highly patterned and are utilized to delineate genera and species, which highlight their significance to the forensic scientists. They can be found anywhere containing moisture but most often encountered in naturally occurring water bodies such as rivers, lakes, oceans, seas and ditches. Diatoms vary in sizes from 2 micrometer to 800 micrometer in length or diameter. Most species are from 10–80 micron in length. When the body (dead or alive) falls in water, the drowning fluid and particles like diatoms and planktons pass down the air passage to lungs. Only a live body with a circulation can transport diatoms from the lung to the other viscera. Diatoms measuring up to 60 microns in diameter are said to enter the pulmonary circulation from the ruptured alveolar walls during drowning. They travel through lymphatic channels and pulmonary veins to enter to the left side of the heart to be circulated throughout the body. Although lungs seem to be the primary channel through which diatoms reach the internal organs, there is also a possibility of diatoms reaching internal organs through alimentary canal. After the passage through the epithelium the diatoms are transported into all parts of the body by the blood stream. Diatoms can be found in a number of organs, but the diatoms test is mostly applied to the bone marrow, the kidneys and the liver. The postmortem extraction and recovery of diatoms is possible because the siliceous extracellular coat of the diatom frustules is resistant to acid digestion, heat and putrefaction. Had diatoms confined themselves to water only, the diagnosis of death by drowning would have been comparatively easy, but unfortunately it is not so and diatoms are almost ubiquitous throughout the world wherever light, moisture, and nutrient are available and there are about 15,000 species of diatoms. Even respired air contains fairly large number of diatom cells and valves. Timperman (1969) made very thorough studies of the diatoms test and concluded that it provides most reliable proof of drowning.[4] This study is attempt to highlight the presence of diatoms in bone marrow and liver in drowning and non-drowning cases in Bikaner region.

Materials and methods

Source of cases:

Cases for the present study were selected from the medico– legal autopsies conducted in the autopsy wing of Department of Forensic Medicine and Toxicology, S.P. Medical College and A.G Hospitals, Bikaner between May, 2012 to December, 2013. Total 1124 autopsies were conducted during this period, out of which 100 cases were selected for examination, 50 cases of drowning and 50 cases of non drowning.

Inclusion criteria of the Cases:

1. All cases of drowning and non drowning of all age group and either gender, irrespective of religion and socio economic status.

2. All cases of drowning in which body was recovered from the water.
3. All cases of drowning and non drowning whether they are identified or not.
4. All cases of drowning in which whether the body were fresh or decomposed.

Related general information like the age, gender, religion, socio economic status, last seen alive and the history about scene of crime etc. of the cases were collected from relatives, eye witnesses, concerned investigating Police officer, Police Panchnamas and filled in relevant proforma.

Exclusion Criteria of the cases:

Highly decomposed bodies of non drowning cases were excluded from the study.

Diatom test by Nitric Acid Digestion:

The sternum were removed and washed with distilled water. A piece of bone was removed with a sharp and distilled water cleaned knife. The marrow was curetted out from the bone and about 5gm of bone marrow tissue was taken in test tube and another about 5gm tissue of liver taken in separate test tube to digest the material. Five times volume of analytical grade concentrated hydrochloric acid and concentrated nitric acid 1:3 ratio was added. The material is left overnight at room temperature to allow digestion. The material was transferred to a centrifuged for 5 minutes at the rate of 3000rpm. The supernatant was removed and deposit was retained, which was further washed with distilled water. This process was repeated three times. After the final supernatant is thrown out, remaining deposit was taken for the final examination and examined under microscope after air drying.[5]

Results and observation

Table 1, Shows the detection of diatoms in sternum was maximum in Canal water drowning (68.75%) and in Water pit drowning (100%) but only 1 case was reported hence statistically insignificant. Diatom detection was minimum (23.52%) among Water tank drowning. In table 2, Shows the detection of diatoms in liver was maximum in Canal water and Pond water drowning (87.50%) except water pit drowning (100%) but only 1 case was reported and hence statistically insignificant. Diatom detection was minimum (70.58%) among Water tank drowning.

In table no. 3 shows the diatoms were detected in 8 (26.66%) cases in 12–24 hours, 7 cases (77.77%) in 1–2 days and 6 cases (85.71%) while time since death in 3–5 days. Diatoms were detected in none of the case up to 12 hours since death in sternum in drowning cases and in table no. 4 Shows only 3 cases (6%) diatom were detected in non-drowning cases. Diatoms were detected in 2 (15.38%) cases while time since death 6 to 12 hours, in 1 (3.23%) case while time since death 12 to 24 hours. No diatoms were detected in other span of time since death.

Table no. 5 shows the detection of diatoms in liver only 23 (76.67%) case while time since death 12 to 24 hours. In 6 (85.17%) cases while time since death between 3 to 5 days and in 1 (100) and in 1(100%) case while time since death 0 to 6 hours or 6 to 12 hours respectively but it statistically insignificant in drowning cases. In table no. 6 shows the out of 50 cases diatom was detected in 13 subjects (26%) in non-drowning cases.

Discussion

The present study was conducted in autopsy wing of Department of Forensic Medicine and Toxicology, S.P. Medical College and A.G. of Hospitals, Bikaner (Raj.) during the period from May 2012 to December 2013. Out of 1124, autopsies during this period 100 (8.89%) cases (50 drowning and 50 non drowning) were examined and shows the maximum incidence of drowning is 32% cases seen in age group of 11-20 years. Momonchand et al[6], Surendra Kumar Pandey[7] and Pranab Chaudhary[8] observed almost similar findings.

Table 1 Showing Site of Drowning and Diatoms Detection in Sternum.

S. No.	Gender	Sternum							
		Canal		Pond		Water Tank		Water Pit	
		No. of Cases	Diatoms Detected	No. of Cases	Diatoms Detected	No. of Cases	Diatoms Detected	No. of Cases	Diatoms Detected
1.	**Male**	11	8 (72.72%)	14	5 (35.71%)	4	0 (0%)	1	1 (100%)
2.	**Female**	5	3 (60%)	2	1 (50%)	13	4 (30.76%)	0	0 (0%)
Total		16	11 (68.75%)	16	6 (37.50%)	17	4 (23.52%)	1	1 (100%)

Table 2 Showing Site of Drowning and Diatoms Detection in Liver.

S. No.	Gender	Liver							
		Canal		Pond		Water Tank		Water Pit	
		No. of Cases	Diatoms Detected	No. of Cases	Diatoms Detected	No. of Cases	Diatoms Detected	No. of Cases	Diatoms Detected
1.	**Male**	11	9 (81.81%)	14	12 (85.71%)	4	3 (75%)	1	1 (100%)
2.	**Female**	5	5 (100%)	2	2 (100%)	13	9 (69.23%)	0	0
Total		16	14 (87.50%)	16	14 (87.50%)	17	12 (70.58%)	1	1 (100%)

Table 3: Shows Detection of Diatoms in Sternum with Time since Death in Drowning Cases.

S. No.	Time Since Death	Total no. of Cases	Diatoms Detected		Total Positive Cases
			Male	Female	
1.	0-6 hours	1	0	0	0
2.	6-12 hours	1	0	0	0
3.	12-24 hours	30	3	5	8 (26.66%)
4.	1-2 days	9	4	3	7 (77.77%)
5.	2-3 days	2	1	0	1 (50%)
6.	3-5 days	7	6	0	6 (85.71%)
	Total	50	14	8	22 (44%)

Table 4: Shows Detection of Diatoms in Liver with Time since Death in Drowning Cases.

S. No.	Time Since Death	Total no. of Cases	Diatoms Detected		Total Positive Cases
			Male	Female	
1.	0-6 hours	1	1	0	1 (100%)
2.	6-12 hours	1	1	0	1 (100%)
3.	12-24 hours	30	12	11	23 (76.67%)
4.	1-2 days	9	4	5	9 (100%)
5.	2-3 days	2	1	0	1 (50%)
6.	3-5 days	7	6	0	6 (85.71%)
	Total	50	25	16	41 (82%)

Table 5: Shows Detection of Diatoms in Sternum of Non Drowning Cases with Time since Death.

S. No.	Time Since Death	Total no. of Cases	Diatoms Detected		Total Positive Cases
			Male	Female	
1.	0-6 hours	1	0	0	0 (0%)
2.	6-12 hours	13	2	0	2 (15.38%)
3.	12-24 hours	31	1	0	1 (3.23%)
4.	1-2 days	4	0	0	0 (0%)
5.	2-3 days	0	0	0	0 (100%)
6.	3-5 days	1	0	0	0 (0%)
	Total	50	3	0	3 (6%)

Table 6: Shows Detection of Diatoms in Liver of Non Drowning Cases with Time since Death.

S. No.	Time Since Death	Total no. of Cases	Diatoms Detected		Total Positive Cases
			Male	Female	
1.	0-6 hours	1	0	0	0 (0%)
2.	6-12 hours	13	4	2	6 (46.15%)
3.	12-24 hours	31	3	3	6 (19.35%)
4.	1-2 days	4	0	0	0 (0%)
5.	2-3 days	0	0	0	0 (100%)
6.	3-5 days	1	1	0	1 (100%)
	Total	50	8	5	13 (26%)

In the present study the diatom detection in sternum with site of drowning was studied. We observed that out of 16 cases drowned in canal, diatoms were detected in 68.75% cases. Out of 16 cases drowned in pond, diatoms were detected in 37.50% cases. Out of 17 cases drowned in water tank, diatoms were detected in 23.52% cases. Maximum diatoms 68.75% were detected in canal and minimum 23.52% in water tank. The diatom detection rate in liver was observed higher that is 87.50% in case of canal and pond water drowning as compared to 70.58% in water tank drowning. This seems to be due to the nature of activities in which they are engaged. The diatom detection rate was observed higher in canal water drowning as compared to the other sites of drowning. The higher detection rate is possible due to the slow flow, shallow depth and higher exposure to sun light which is favorable for the growth of diatoms. The diatom detection rate was observed higher that is 87.50% in case of canal and pond water drowning as compared to water tank drowning (70.58%) this again is possibly due to the less exposure of water tank to direct sun light which is unfavorable to the growth of the diatoms.

The diatom detection in sternum of total cases with time since death was studied; it was observed that up to 6 hours of time since death, no diatom was detected. Maximum diatom detection 75% was observed when time since death was between 3-5 days. No data from previous work in this regard was available to us for comparison. When the diatom detection in liver of total cases with liver since death was studied; it was observed that diatom detection in liver does not show a definitive pattern with time since death.

In the sternum in drowning cases the diatoms were detected in 22 (44%) cases out of 50 cases. The detection rate of diatom in liver was almost double is compare to the

sternum as the liver has rich blood supply as compared to sternum and also has portohepatic circulation.

The detection of diatom in sternum of non drowning cases was studied with time since death. Out of total 50 cases the diatoms were detected in sternum of 3 (6%) cases of non drowned. The detection of diatom in liver of non drowning cases was studied with time since death. Out of 50 cases diatoms were detected in 13 (26%) cases.

The detection rate of diatom is almost four times in liver as compare to sternum in non drowning. This detection of diatom in liver and sternum is in accordance with Hendey (1973)[5] who gave explanation for the presence of diatoms in non drowned cases as by eating raw fruits or vegetables and shell fish; large amount of diatoms present in materials used for manufacturing of buildings like cement, paint, paper etc, and the person might have inhaled in that atmosphere and may have such diatoms. They can also penetrate the intestinal lining and gain excess to blood stream. The findings are consistent with N. Foged[9] who recovered diatoms from four non drowned cases. The findings are consistent with La Yen Yen[10] who reported abundance of diatom frustules in the sample of cooked non vegetarian food stuff.

The findings are consistent with Pollanen et al[11] as they recovered diatom from femoral bone of 28% of fresh water drowning and 12% of domestic water drowning. They conducted that the test was highly supportive in cases of drowning but has low sensitivity. According to Svetislav et al[12], they recovered diatom cells from the organ of a confirmed non drowning case and they concluded that overall analysis of diatom cell abundance in organ should have quantitative figure.

The findings are in accordance with J.J. Tailor[13] who described a case in which long term repeated exposure of the body to diatom containing water laid to diatom finding in tissues.

Apurba Nandy[14] as they mentioned presence of diatom in body tissues as suggestive proof of ante mortem drowning. The findings in our study are in favour of Richar Shepherd who stated that this test now so unreliable that it should only be used, and the result interpreted with great circumspection.

Conclusion

The present study to conclude that diatoms detection in both drowning and non drowning cases do not show a definitive relation with time since death. Due to the low sensitivity & specificity of diatom test in drowning as observed during this study, the diatom test is not a gold standard test for drowning.

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