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A study of significance of diatoms test in drowning vs non-drowning cases

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Abstract
Background: Drowning is a form of asphyxia due to submersion or immersion in liquid, which are mostly accidental. Death by drowning is difficult to determine and often done by eliminating other causes of death. One of the most important criteria in the study of drowning is the search for a sensitive and specific test. The diatoms are major group of eukaryotic algae and stand as the strongest biological forensic evidence.
Aims & Objectives: The study aim to compare the significance of diatoms test in drowning and non drowning cases.
Methods: The study carried out in the Dept. of Forensic Medicine, Guntur medical College, Mortuary, Government General Hospital, Guntur during the year January 2017 to December 2017.After selection of the cases during the post-mortem examination the tissues or organs are taken and diatoms test is done as per the procedures in drowning and non drowning cases. The various types of diatoms are studied in detail. The sample of drowned water was also examined for presence of diatoms. A comparison is made for similar types of diatoms to get the significance of diatom test. The relevant water samples in the particular rural locality also are examined and compared in non-drowning cases.
Results: In the present study out of 40 cases, 35 are true positive and 5 cases were true negative. We found 20 non drowning cases 18 were true negative and 2 are false negative cases were recorded in our study.
Conclusions: Diatom test in drowning is significant even though occasional diatoms may be recovered in lungs and stomach mucosa of non-drowning cases. This diatom test is very valuable in drowning deaths especially in decomposed states as other findings of drowning may be lost or obscured due to decomposition.

Keywords: Diatoms. acid digestion test, lungs, stomach mucosa

Introduction
Death by drowning is defined as a death due to submersion in a liquid and the mechanism in acute drowning is hypoxemia and irreversible cerebral anoxia. Although there are some typical signs of drowning known, it is still hard to determine a death by drowning when the post-mortem signs are impossible to find in case of deceased and therefore a great number of tests have been proposed to allow confirmation of death by drowning of a victim. Diatom test is one of these tests and works as an important tool in diagnosis of death due to drowning, it can be used to discriminate between drowning and non-drowning cases. Detection of diatoms in tissues may contribute to diagnosis of drowning, therefore an efficient method of extraction and microscopic examination of diatoms from tissues is necessary, however, it is important to remember that the absence of diatoms does not immediately rule out drowning; the test does not prove the negative, and a thorough investigation is always required.
The cause of death was attributed to drowning as diatoms can be carried to the distant parts of the body if the person was alive when entered into the water. These results were also supported by many studies done from time to time (Timperman, 1962; Peabody, 1978; Jakhar *et al*., 2013) [13, 6, 16, 14]. Five cases were found to be negative for the above mentioned test as no diatom species were detected from the bones and visceral exhibits of the suspected drowning victims. However, diatoms were detected from the water samples of all the three sites. These persons were dead already. So the reason of death was other than drowning because diatoms could not be circulated in the distant body organs in dead person.

Thus diatoms can be used as an important tool in the diagnosis of drowning cases. Various workers supported the reliability of the diatom test (Ludes *et al*., 1994; Pollanen, 1998; Vinayak *et al*., 2010; Verma, 2013) [17, 18]. Diatom test is frequently used in forensic science laboratories in India indrowning diagnosis, but at the same time, a large pool of forensic experts is confused about the practicality and reliability of the test for ante-mortem drowning detection.

Also, most of the diatoms are observed under light microscope as availability of an electron microscope is veryfew in the country. Therefore, the only comparison of shapesand sizes of different diatoms never confirmed the species of diatoms (Kumar *et al*., 2012) [19].

Hence in the present study, the main objective is to study;

1. To detect the diatoms in various organs and tissues in all drowning cases.
2. To compare and contrast the various types of diatoms in the tissues with the sample of water
3. To detect the presence of diatoms in deaths due to non-drowning cases.
4. To analyse the significance of false positive and false negative cases with respect to diatoms.

Materials and Methods

Type of the study

Prospective comparative analytical study.

Duration of the study

1 Year (From January 2017 to Dec 2017).

Place of the study

Department of Forensic Medicine & Toxicology, Guntur Medical College, Guntur, AP

Inclusion criteria

- All the deaths due to drowning brought to the mortuary, GGH, Guntur for post-mortem examination.
- Selected cases of deaths due to non drowning cases of rural areas brought to the mortuary, GGH, Guntur.

Exclusion criteria

Cases other than drowning are not included as confirmed by history and police investigation. Bodies with multiple fractures were excluded from the study.

Collection of water sample

Samples of water are collected as soon as possible after the body is discovered. Approximately 1 litre of water is collected. Water samples are placed in clean container and labelled. A diatom free formalin solution is added to water (1ml 2% formalin in water) to inhibit microbe growth indefinitely so that the same can be stored at room temperature.

Methodology for Postmortem analysis of drowning cases

After post-mortem of dead bodies, internal organs (lungs & stomach mucosa) along with the water samples (from where the dead bodies were recovered) were collected. In all cases samples are put into different jars. 50 ml of nitric acid was added in each jar containing the lungs& stomach mucosa. Samples were left undisturbed overnight and boiled for half an hour on next day. A clear yellow solution was obtained with a fat layer at the top. The fat layer was discarded and the remaining samples were centrifuged at 4000 rpm for 10 minutes. Supernatant was discarded and washed with distilled water and microscopic slides were prepared. Comparison of diatom species found in organ sample and water sample was done and correlations were drawn out accordingly (Hürlimann *et al*., 2000) [7].

Statistical analysis

The collected data analysed with suitable statistical tool and to find out the significance of the results.

Results

60 selected cases were studied. Among them 40 cases are drowning and 20 are non drowning cases. Out of the 40 samples, 35 showed positive diatom test and the rest showed negative results. Out of the 20 cases, 18 cases showed true negatives and remaining two cases showed false negative. In the present study, most of the cases belongs to illiterate 25 (62.5%) followed by primary education 20%. Few number of cases were observed in graduation and higher graduation cases. The present findings showed most of the cases were belongs to low income group 20 (50%), followed by middle income group 15 (37.5%). In the present study, most of the drowning case were examined in both married and unmarried cases, here there was no significant difference in the results. In drowning cases males were more prone than the female. We observe drowning cases only in elder males than the younger ones.

In our study, it is observed that in only 12.5% cases there were injuries over the body, while in most (87.5%) of cases there was no injury found over the body. Out of 5 (2.5%) cases, major type of injury was found only 1case which is sufficient to cause of death, while rest4 (10%) cases had minor injuries. Regarding animal or aquatic animal bites over the body, we found 30% cases having animal bites while most (70%) cases did not have such bites. Dead bodies were found floating in more than half (52%) cases, while other (48%) were in submerged position when recovered from the site. Presence of watery fluid in pleural cavity was found in one third of the cases (75%). While in rest (25%) cases, there was no fluid in pleural cavity.

Table 1: Distribution of drowning cases according to age and sex

Age group	Male		Female		Total	
	No	%	No	%	No	%
<15	06	15	04	10	10	25
15-30	14	35	06	15	20	50
31-44	06	15	02	05	08	20
>45	02	05	00	00	02	5
Total	28	70	12	30	40	100

Table 2: Occupation

Occupation	No	%
Student	20	50
Laborour	08	20
Farmer	04	10
Housewife	04	10
Government employ	01	2.5
Unemployed	03	7.5

Table 3: Educational Status

Educational status	No	%
Illeterate	25	62.5
Primary Education	10	20
Any graduation	03	7.5
Higher education	02	5

Table 4: Socio-economic status

Income status	Number	%
Low income group	20	50
Middle income	15	37.5
High income	05	12.5

Table 5: Marital status

Marital status	No	%
Un married	15	37.5
Married	16	40
Divorce	05	12.5
Widow	04	10

Table 6: Distribution of drowning cases according to associated injury, animal bite and other findings (N=40)

Findings		
Associated body injury	**No**	**%**
Major	1	2.5
Minor	4	10
No injury	35	88.75
Animal & aquatic animal bite		
	No	**%**
Yes	12	30
No	28	70
Fluid in pleural cavity		
	No	**%**
Yes	30	75
No	10	25

In the present study out of 40 cases, 35 are true positive and 5 cases were true negative. We found 20 non drowning cases 18 were true negative and 2 are false negative cases were recorded in our study.

Table 7: Type of Diatoms fond in the drowning cases

Diatom species	Status
Coconis	+++
Cuniate	++
Centric	++++++++++
Naviclond	++
Small pennicate	+++++
Hetero polar	+++
Dorsi ventral	++++
Nitzschia	++
Elongated	++
Filamentous	++

In the present study, the major diatoms found in the drowning cases were centric in shape which followed by small pennate type of diatoms were observed in most of the drowning cases. In non-drowning cases we observed different type and shapes of the diatoms.

Discussion

Out of 60 cases, 40 drowning cases and 20 non drowning cases were studied. A study found rural dominance in contrast to among identified cases, maximum numbers of victims (45%) were unmarried. The reason may be that they do not have sufficient maturity and liabilities of their family which act like an inspiration to live and behave carefully and secondly because of more stressful and ambitious student life. Both together can lead to suicidal tendency and accident prone behaviour. More than half (50%) cases belonged to 15-30 years of age group which is also observed by others. This is more active period of life having great fluctuations of emotions, violent and arrogant nature and frequent participation in water activities. Also a tendency to be more reckless could explain the high proportion of victims in this age group. Male preponderance (70%) seen in the study was comparable to other studies. Studies suggest that males have higher drowning rates than females due to increased exposure to water and riskier behaviour, such as swimming alone, drinking alcohol before swimming alone and boating. Literacy rate was 62.5 percent, but higher education like graduation was seen only in 5% cases. This was well correlated with higher proportion of lower socio-economic class (59.1%) and occupation wise major proportion of students (50%) and labours (20%). Similar findings were reported by others. Lower level of literacy and low socioeconomic status are considered major risk factors for drowning all over the world (Chowdary *et al.*)

Postmortem findings

The majority of the autopsy findings are related to asphyxia and have no specific link to drowning. The signs of drowning depend on the delay in recovering the body and on the development of the putrefaction phenomenon which alter the positive signs of drowning.

One of the signs of drowning would be large amounts of froth present around nostrils and mouth in freshly drowned bodies. This froth is also present in the upper and lower airways.

It is admitted that lung weights are higher in drowning cases but it was shown that normal weights are possible in the drowning cases after cardiac arrest reflex or vaso vagal reflex.

Most of the cases show bleaching appearance (25), soddening appearance (22), whitish pale discolouration (5), puckering of skin (8), cutis anserina (8) cases were observed.

Role of diatoms has always remained significant in solving the drowning cases. In cases, where the cause of death cannot be ascertained by conventional postmortem examination in those cases presence of diatoms in lungs and stomach mucosa plays an important role in determining whether the death is due to drowning or not.

Analysis of diatoms present in the body tissues like lungs, liver, spleen, blood and bone marrow has been undertaken as supportive evidence in drowning cases. The theory behind the 'diatom test' is that when any person drowns, the diatoms present in that water will reach to the lungs and some of them because of their size penetrate into the alveoli. If the heart is still beating, the diatoms that have entered into the blood stream travel around the body and may lodge in distant organs such as the kidneys, brain and bone marrow before death (Auer, 1991; Pollanen, 1997) [4, 5].

The presence of diatoms in the internal organs most likely confirms the ante-mortem drowning. If there is any doubt about the drowning site, then water sample from the putative

site of drowning can be collected and analysed to determine the similarity of different species of diatoms in the water and the body.

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 whether the drowning 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.

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. (Singh *et al.*)

In the present study, Stomach mucosa and lungs studied in non-drowning cases showed presence of diatoms. Out of 20 non drowning cases, only 10 cases showed diatoms in lungs. The diatoms seen in lungs were very less in number that only one or two were seen in one full slide. Mostly these were centric or small pinnate types. The diatoms in lungs showed pennate, centric and naviculoid in abundance.

Conclusion

During the present study, it is concluded that among 60 sample cases, 40 cases were positive (death due to drowning) while 20 cases were non drowning cases. The present results revealed that 35 cases show positive for diatoms. And in 20 non drowning cases, only 2 cases show diatoms which is insignificant. Hence the diatom test is considered as reliable test to determine whether the drowning is antemortem or post mortem in nature.

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