Diatom Test- Past, Present and Future: A Brief Review

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ABSTRACT

Every test or methodology has to go through litmus test with passage of time. If proved wrong, it loses its credibility, but if it is found correct again and again, its importance and application increase over the time. History of application of diatom test as an important tool in diagnosis of drowning death is only a century old. As typical features of ante-mortem drowning disappear very rapidly with commencement of putrefaction, diatom test had gained the importance as gold standard for diagnosis and confirmation of drowning deaths. Drowning, as cause of death had been assigned in many cases based on diatom test in the past. Claims of detection of diatoms in non-drowning cases in the last five decades have raised doubts about the reliability of diatom test. Presently, forensic pathologists all over the world are divided over reliability of diatom test as a diagnostic aid. However, advent of newer methodology such as Nuclear Magnetic Resonance (NMR) and Inductively Coupled Plasma Hyphenated Technologies, Atomic Force Microscopy, Fluorimetry, Automatic Diatom Identification and Classification (ADIAC) have raised the hope for survival of Diatom test in the future.

Key Words: drowning death, diatom test, ADIAC, Atomic Force Microscopy

INTRODUCTION

Diatoms are unicellular plant that has most distinctive features of crystalline extracellular coat or frustules composed of silica and having unique patterns of symmetry and microstructure. There are more than 200 genera and 100,000 species of diatoms.[1] Although diatoms are ubiquitous in nature, they are most frequently detected in naturally occurring bodies of water. Some species have preference for water of specific salinity. However, some diatoms are more frequently found in soil and puddles than in lakes.[2]

Since the detection of diatom in lungs in a victim of drowning death by Revenstorf in 1904, diatom test has been considered as an important tool in diagnosis and confirmation of the death due to drowning.[3] In the past, final opinions regarding the cause of death of individuals recovered from fluid or water medium had been given on many occasions solely on the basis of diatom test. Typical fine, white colored lathery froth in the mouth, nostrils and respiratory passage, typical features of drowning lungs and other associated positive proof of ante mortem drowning disappear very rapidly with development of putrefaction. Furthermore, dead bodies recovered from water may have died due to any cause other than drowning or just can be method of disposal of the dead body for poverty, custom or hiding crime. In such situations, detection of same type of diatoms both in drowning medium and body tissues was thought as gold standard to diagnose drowning death as well possible place of drowning. However, claims of detection of diatom in non-drowning cases in the last five decades have raised doubts about its reliability. With advent of newer technology in the field of forensic limnology has raised the hope for revival of Diatom test.

Brief history of diatom test

Diatom was first detected in lung fluid by Hofmann in the year 1896.[4] However, Rovenstorff had successfully utilized it to solve a case of a drowning mystery in 1904. The method of its extraction was improved by acid digestion of the tissues.[5] Incze successfully detected diatoms in blood and parenchymal organs in 1942.[6] Tamasaka detected diatom in bone marrow in 1949.[7] In the 1960's and 1970's, Timperman using large series of drowning cases, provided evidence for the validity of diatom test.[8]

Necessity of Diatom Test

All the dead bodies recovered from water medium are not necessarily died of drowning. They could have died either of a natural disease or of a trauma before entering, or while inside the water medium.[9] Thus drowning can be ante-mortem or post-mortem. Though decomposition starts late in the water medium than air, most of the submersed dead body are detected only after its flotation to water surface, caused by decomposition. Even if a non-decomposed body is recovered from water, it undergoes rapid putrefaction, till it reaches morgue. Once decomposition sets in, almost all the important features of ante-mortem drowning vanishes very rapidly. Even the general asphyxial features are masked by putrefactive colour changes. Ante-mortem injuries during the process of drowning, post-mortem injuries during flow of the dead body in water medium or post-mortem injuries inflicted by aquatic animals can only add further problem to diagnose cause and manner of drowning. Circumstantial histories are often found lacking or misleading in these cases. In the above circumstance, no conclusive evidence is left in the dead body to assign a proper cause of death. Thus diatom test raises hope for diagnosis and confirmation of ante-mortem drowning. Identification and comparison of diatom species further raise the hope for diagnosis of possible site of drowning. Due to plenty of diatoms are found in water medium, due to non-disputed physiology behind its entry into circulation during the process of drowning, and due to its easy applicability without requiring sophisticated laboratory set-up, it was thought to be "gold standard" in diagnosis of drowning death until the recent past.

Methodology applied for the Diatom test

Tissues commonly employed in the Diatom test include lungs, liver, kidney, brain, and bone marrow. Of these, bone marrow is reported to be the best as it proves the hypothesis of ante-mortem drowning as well as it is least affected by contamination during post-mortem submersion of the body, or preparing process of Diatom Test.[5,9]

Commonly used methods for digestion of human tissues for extraction of diatom are acid digestion test, using nitric acid; soluene-350, especially in case of sea water drowning; Ashing method and enzymatic tissue digestion, especially using proteinase K.[10]

Several procedures have been developed to replace the original acid digestion method for extracting frustules from human tissues. Most of the methods rely on alternative chemical or physiochemical means to solubilize the tissue. Methods that have been particularly useful are enzymatic digestion of tissues with proteases such as proteinase K. Strong anionic detergents such as sodium dodecyl sulphate and non specific tissue solubilizers have also been shown to be effective for frustule extraction. Simple centrifugation at 3500 RPM for 30 minutes each at different stages is usually used to isolate diatoms from extracted tissue suspensions. Membrane filtration and gradient centrifugation have also been advocated. Although many methods have been suggested as alternatives to acid digestion, there is no evidence for improved yield of frustules. Little is known about the centrifugal properties of frustules, in particular, the centrifugal forces that gives optimal quantitative isolation. This may be relevant since small pennate diatoms may be more difficult to isolate, using differential centrifugation, than larger genera such as Navicula. New perspectives are provided by the polymerase chain reaction (PCR) for identifying diatoms by means of primers for chlorophyll related genes.[11,12]

Controversies surrounding Diatom test

Like magical hydrostatic test, diatom test has become one of the most controversial and debated test in the present scenario. If diatoms were only found in human organs after death by drowning, then their presence alone would be the answer to many different questions in its diagnosis. However, it cannot be denied that diatoms may find access to the human tissues through several pathways, through the lungs, by breathing contaminated air or through various foods that have high diatoms content. The relatively common occurrence of diatoms in human tissue led to a strong divergence of views concerning the value to be placed upon diagnostic significance of diatoms found in subjects died from drowning and the diatoms found in subjects known to have died from causes other than drowning (non-drowning). The crux of the matter is the possibility that non-drowned subjects may contain diatoms. Otto (1961) reported on workers in Kieselguhr industry who had contracted silicosis through continuous inhalation of diatoms in the lungs, without of course, having drowned.[14] Spitz (1963) and Peterson (1963) showed diatoms to be present in the major organs of non-drowned subjects, and came to conclusion that the diatom method was worthless.[15,16] Porawski (1966) also found diatoms in the lungs, kidneys and bone marrow of non-drowned subjects.[17] Koseki (1968) reported their occasional presence in the lungs (and rarely in the liver and kidney) of non-drowned bodies.[18] Schellmann and Sperl (1979) had found diatoms in the bone marrow of 15 out of 16 non-drowned subjects.[19] These finding suggested that the presence of diatoms in vital organs is not a reliable indicator of death due to drowning. If these results were consistently found then the diatom test would have been shown the door by now. However, Tamaska (1949) found no diatoms in the bone marrow of seven subjects who were shot before entering the water. Muller (1963) did not find diatoms in the liver of 30 non-drowned bodies.[20] Neidhart and Greendyke (1967) failed to detect diatoms in the organs of 15 non-drowned bodies.[21] Peabody AJ (1977), Ludes B et al., (1994) and Pollanen MS (1997) strongly supported diatom test in diagnosis of drowning.[22,23] Pollanen MS claimed that diatoms found in non-drowning cases could have caused by contamination during various process of autopsy and diatom test. Bortolotti F et al., (2004) advocated the use of Environmental Scanning Electron Microscope (ESEM) over light microscope for better diagnosis of diatom.[13] WANG Lei et al., (2004) advocated Maximum Value of Diatom aspirated into the lung (number of diatoms per tissue in gram) to differentiate drowning and non-drowning deaths.[24] Most of the workers advocated proper identification and matching of species of diatoms both in body tissues and drowning water medium for diagnosis of drowning.

Present status and Problem of Diatom test in India

To demonstrate drowning unambiguously as the cause of death remains a difficult issue in current forensic practice.[11] Forensic experts across the country are also divided and confused over the utility and reliability of Diatom test for diagnosis of ante-mortem drowning. It is now proven fact that diatoms are also seen in body tissues of non-drowning deaths. Hence, mere detection of diatom in body tissue cannot be called as proof of ante-mortem drowning. Availability of scanning electron microscope is very few in the country. Most of the diatoms are observed by only light microscope. Mere comparison of shapes and sizes of diatoms never matches species of diatoms. As there are more than 10, 000 species of diatoms, identification of species is not an easy task at all even for the most experienced limnologist, which can be numbered in this country.

Newer hope for revival of Diatom test

Over the period, since the detection of diatom in drowning death, the methods adopted for diagnosis of drowning remains almost unchanged. However, advent of newer methodology such as Nuclear Magnetic Resonance (NMR) and Inductively Coupled Plasma Hyphenated Technologies, Atomic Force Microscopy, Fluorimetry, Automatic diatom identification and classification (ADIAC) have raised the hope for revival of Diatom test in the future, though their availability to all parts of the world may takes longer duration.[25]

Nuclear Magnetic Resonance (NMR) and Inductively Coupled Plasma Hyphenated Technologies

NMR is a highly technical and precise method of measuring spatial molecular orientation in organic, organo-metallic or bio-chemicals as found in nature. Currently, 2-D and 3-D NMR instruments techniques have proven effective in a diversity of matrices and sample types. It can be used to identify and classify unknown species and genera of diatoms. By coupling NMR with Liquid

Chromatography (LC) and Mass Spectrometry (MS) i.e., hyphenated techniques using selective pre-analytical workup of matrices, even fractions of diatom can be classified, also their chemical content reflective of aqueous environment where they present originally. NMR may be positioned effectively by imaging differences between normal bone marrow as distinguished from the presence of diatomaceous particles, without having the need for tissue processing.

Atomic Force Microscopy (AFM)

This technique is derived from STM (Scanning Tunneling Microscopy). Individual atoms and other microscopic samples can be resolved on or in insulated surface such as water. The advantage of this approach over other direct-measuring techniques is that it allows minute solid samples to be measured and displayed within water or another liquid medium. AFM can detect the features of specific and individualized atoms and scan an object as large as 8" long and 0.5" in diameter, well within the size of even the largest diatom. It has advantage over NMR as it increases the applicability of AFM to any sample taken independent of source where the presence of diatoms is suspected. Because of its ability to scan into an object, both in vertical and horizontal axis, AFM allows three-dimensional imaging, further increasing its accuracy. Minimal sample preparation, extremely high sensitivity, and outstanding flexibility to diverse sample (live cells and membranes to bone and cartilage), places this advanced technology highly desirable.

Fluorimetry

Fluorimetry can be used to better locate and isolate diatoms in a sample of bone marrow or other tissue by luminescent properties. It can differentiate diatoms found at suspected site of drowning from other diatoms found in nature, by incorporating specific fluorescent tags.

Automatic Diatom Identification and Classification (ADIAC)

Automatic diatom identification and classification project has been founded in 1998 by Vision laboratory of University of Algarve. It is concerned with applying image processing and pattern recognition tools toward the identification of diatoms by computer, very similar to Automatic Fingerprints Identification Systems (AFIS). It has purpose to develop appropriate image databases and analytical methods for the automated identification of diatoms. Unlike the conventional methods, this system will be able to more quickly pinpoint a greater number of diatoms in a given sample. This technique can be accurately used to recognize the rheology of a small number of diatoms from a species pool of over 10,000.

CONCLUSION

Currently, the credibility of diatom test as a diagnostic aid to confirm drowning deaths is put under scanner. However, with the advent of newer technologies such as Nuclear Magnetic Resonance (NMR) and Inductively Coupled Plasma Hyphenated Technologies, Atomic Force Microscopy, Fluorimetry, Automatic Diatom Identification and Classification (ADIAC), the Diatom test is on its way for a revisit.

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