PROGRESS IN MEDICAL SCIENCES, 2020
VOL 4, NO. 1, PAGE 1 – 6
DOI: doi.org/10.47363/PMS/2020(4)119

REVIEW ARTICLE

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A review on unconventional methods for the diagnosis of drowning

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ABSTRACT

Drowning is one of the most complex pathophysiological phenomena in forensic medicine. Till date, no accurate diagnostic test exists to diagnose death due to drowning. This is probably due to the close relation of drowning death with death caused by asphyxia. Although in the recent years, there have been various advances in the methods to diagnose drowning accurately. Among the conventional methods, the estimation of various ions from vitreous humor, pericardial fluid, pleural effusion fluid, and fluid from sphenoid sinus have been made more accurate by using state of the art equipments. The correlation with lung weight has also shown great results in improving the accuracy of diagnosis of drowning cases. Biomarkers like pulmonary surfactants and aquaporins have also shown great potential in developing a screening test to diagnose drowning death. Receptor for Advanced Glycation End Products (RAGE) is another such new biomarker. Its mRNA expression levels have shown great potential in diagnosing drowning deaths. These biomarkers as well as the modified conventional methods, such as impedance spectroscopy, postmortem computed tomography, and electrolyte estimation from various bodily fluids have shown the potential to increase the accuracy of diagnosing drowning deaths, manifold.

ARTICLE HISTORY

Received October 07, 2019
Accepted December 16, 2019
Published January 17, 2020

KEYWORDS

Drowning;
diagnosis;
forensic

Introduction

In the field of forensic medicine, there is no reliable method to accurately diagnose a case of drowning. This is due to the complex pathophysiology of drowning. It depends on various factors, such as asphyxia and damage to lungs, due to aspiration of the immersion fluid; change in blood constituents as a result of drowning; subsequent change in electrolyte levels in the body as well as metabolic changes [1–4]. There is also scarcity of signs, needed to accurately diagnose cases of drowning [5–7].

There are many morphological changes associated with drowning. These are mainly caused by liquid penetration into the airways. The most common changes are frothy liquid in the airways, external foam, and lung overexpansion [8–11]. But, these changes can also be found in some end-stage pathological conditions. So, it's very difficult to distinguish drowning specific changes. On top of that, these changes can only be assessed when the body is not affected by putrefaction.

Globally, drowning constitutes about 7% of all injury-related deaths (WHO, 2010). According to the WHO Global report on drowning, Preventing a leading killer, published in 2014; around 372,000 drowning deaths occur worldwide each year. Among children and young people, drowning is one of the leading causes of death in every region of the world [12]. Drowning deaths are very common in India, especially in the cases of unnatural deaths. This is due to the fact that it is a preferred method of committing suicide among Indians. 29,903 deaths (6.6% of total accidental deaths) were reported due to drowning in 2014 as per the National Crime Records Bureau, MHA Govt. of India NCRB (2014) [13].

At present, several parameters are judged to diagnose drowning. These include past medical history of the deceased; circumstances before death; thorough internal and external examination of the body, as well as biochemical analyses. Another important aspect for diagnosing drowning is the exclusion of other causes of death [9]. Thus, in order to detect drowning accurately, an ideal diagnostic test needs to be established. In this regard, the following advances have been made.

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Methods

Here, we have systematically reviewed the different articles published from 1995 to 2019 for this purpose from different databases of PubMed, Cochrane Library, etc. The keywords related to the study aim and included in the search string were: drowning, diagnostic tests, biomarkers, and forensic diagnosis. The aim of this article is to highlight newer methods that can be used to diagnose the cause of death in case of drowning.

Combined lung weight

According to the study conducted by Maeda et al. [14], the combined weight of lung tissue and pleural effusion fluid was greater in case of salt water, brackish water, and fresh water drowning in comparison to acute cardiac deaths. In case of salt water and fresh water drowning, it was greater in case of the former.

Modification of diatom test

The diatom test has been one of the main tests for diagnosing drowning for a considerable amount of time. But, the study by Spitz, Peterson, Porawski, Schellmann, and Sperl showed that the accuracy of the test is debatable. They showed that diatoms can be present in the major organs of non-drowned persons also. Such presence may be caused by contamination during autopsy, as suggested by Pollanen [15].

Currently, the detection of diatoms is done by a light microscope. But, it fails to identify the smallest diatoms, thereby decreasing the accuracy of the test. In order to identify the diatom fragments, Scanning Electron Microscope and Environmental Scanning Electron Microscope can be used as they show a much better resolution [16].

Automatic Diatom Identification and Classification is another new method which can increase the accuracy of the diatom test. It uses the same concept of Automatic Fingerprints Identification System. Its goal is the automatic identification of diatoms using computerized analytical methods from appropriate image databases. In comparison to the conventional methods, it will result in the faster identification of greater quantity of diatoms in a sample [17].

Pleural effusion and pericardial fluid

The quantity of electrolytes, such as sodium, potassium, and chloride, in pleural effusion has been used to differentiate between seawater and freshwater drowning as well as to determine the postmortem interval [18,19].

According to the study conducted by Matoba et al. [20], the concentration of Na or Cl lesser than 65 mEq/l is a diagnosis of freshwater drowning.In case of seawater drowning, the diagnosis can be made if the concentration of Na is ≥ 175 mEq/l, or that of Cl is ≥ 155 mEq/l, or that of Ca is ≥ 16 mg/dl, or that of Mg is ≥15 mg/dl.

The use of SUM (Na + K + Cl) as a modified tool for the diagnosis of drowning has also been suggested by many researchers. In the study done by Yajima et al. [21], 21 freshwater drowning cases, 32 seawater drowning cases, and 43 non-drowning cases with pleural effusion were evaluated. There was significant difference of SUM (Na + K + Cl) between the groups (188.8 ± 33.2, 403.5 ± 107.9, and 239.3 ± 21.7 mEq/l, respectively). According to this study, freshwater drowning is suggested by a cut-off value of <195.9 mEq/l and seawater drowning is suggested by a cut-off value of >282.7 mEq/l.

Maeda et al. [14] found the Na, Cl, Ca, and Mg levels in left cardiac serum and pericardial fluid higher in case of seawater drowning. They also found an inverse correlation between pericardial fluid Na and Cl levels and lung weight in case of freshwater drowning.

Vitreous humor

According to the study by Cala et al. [22], Na and Cl levels of value ≥ 284 mmol/l suggest seawater drowning [22]. Various recent studies have shown that the postmortem level of Na and Cl in vitreous humor was elevated in saltwater drowning when the immersion time was less than 1 hour. Also, these studies have shown that this change was due to drowning and not immersion [23–27].

Fluid from sphenoid sinus

According to the study conducted by Tanaka et al. [28] in 2015 using Energy-dispersive X-ray spectroscopy, the liquid taken from sphenoid sinus showed Cl and Br levels below quantification limit in case of freshwater drowning. Other studies by Matoba et al. [29] and Hayakawa et al. [30] have tried to differentiate between sea water and fresh water drowning by analyzing Na, K, Cl, Mg, and total protein. They showed that analysis of Na, K, Cl, Mg, and total protein level in sphenoidal fluid has the potential to differentiate between sea water and fresh water drowning

Pulmonary surfactant

In case of surfactant specific proteins, Surfactant-specific proteins (SP)-A and SP-D are hydrophilic and SP-B and SP-C are hydrophobic. In comparison

to other asphyxial deaths, drowning cases showed higher SP-A1/A2 ratio [8].

Miyazato et al. [31] showed that in case of immunohistochemical detection, all these proteins except SP-A have no significant difference. In case of drowning, mechanical asphyxia, fire fatality, and acute cardiac deaths; the SP-A and SP-D mRNA levels were less in comparison to their respective levels in hypothermia and injury. Also, higher quantitative levels of TNF-α, IL-1β, and IL-10 mRNA were found in case of drowning.

Kamada et al. [32] using enzyme immunoassay showed that SP-D levels were increased in both seawater and freshwater drowning cases. But the mean concentration was higher in seawater drowning cases in comparison to the freshwater ones.

Aquaporins

AQPs are small (~30 kDa/monomer) water transporting proteins found in humans. They are homologous in nature. AQP5 is found in the lungs and AQP2 is found in the kidneys. Their expression can be used to differentiate between freshwater and saltwater drowning [33,34].

The study conducted by An et al. [34] showed that the AQP4 expression in brain could be used to distinguish freshwater drowning from seawater drowning. In the study, immunohistochemistry was used to detect intracerebral AQP4 expression. It was seen that the AQP4 expression in brain was higher in freshwater drowning cases in comparison to the seawater ones.

According to the study by An et al. [35], the AQP2 expression in kidney was seen using immunohistochemistry. AQP2 expression was higher in seawater drowning cases in comparison to the freshwater ones.

The study by Lee et al. [36] showed that mRNA expression of AQP5 showed higher levels in drowning cases compared to other groups. This can be attributed to the fact that AQP5 in type I cells mediate osmotically driven transport of water across the cell membranes [37,38].

Postmortem computed tomography

Postmortem Multislice Computed Tomography (MSCT) is used to measure the density of different body parts in Hounsfield Units (HU) in order to characterize the constituents. In case of freshwater drowning, MSCT can detect frothy fluid in the trachea; nodule shaped ground glass opacities in the lungs; bronchospasm; hemodilution; fluid and sediments in paranasal sinuses, ear and stomach; varying level of hemi diaphragm [39–43].

The study conducted by Christe et al. [39] showed that in case of freshwater drowning, the dome of the diaphragm was located at a lower level (fifth anterior rib level) in comparison to the non-drowning group (fourth anterior right rib level). Also, the broncho-arterial coefficient was 0.84 in drowning and 1.04 in non-drowning cases, thereby showing a significant difference. Hemodilution was lower in case of the drowning cases (50 HU) in comparison to the non-drowning case (64 HU). The drowning cases also showed more aspiration into the trachea and main bronchi along with greater distention of stomach in comparison to the non-drowning cases [39].

The study by Van Hoyweghen et al. [44] showed that only the right hemi-diaphragm height varied between drowning and non-drowning cases.

Impedance spectroscopy

Impedance spectroscopy detects tissue composition by using the electrical properties of the biological tissues. The study conducted by Shiwei et al. [45] showed that the impedance of lung tissue was lower in drowning cases when compared to the cases of postmortem submersion. This attributed to the greater quantity of fluid in lung tissue of drowning victims. In between seawater and freshwater drowning, the impedance was lower in case of seawater drowning cases.

Rage

It is a transmembrane receptor which recognizes patterns. It is a member of the immunoglobulin superfamily. It is found in all cells but in low levels. Its expression is most abundant in the lungs [46]. Secretory RAGE (sRAGE) and membrane RAGE (mRAGE) are the two isoforms of RAGE. sRAGE is detected at 29 kDa in Western blot and acts as a decoy receptor due to lack of transmembrane domain [47]. mRAGE is detected at 45 kDa in Western blot. Its expression is most significant in airway epithelium. It is also found in the smooth muscles of this area in case of chronic obstructive pulmonary disease [48].

The study conducted by Lizotte et al. [49] has shown that the RAGE is a sensitive biomarker for detecting lung diseases and drowning. Another study by Lee et al. [36] has shown that the RAGE expression was significantly higher in case of seawater drowning in comparison to freshwater drowning. The study by Cho et al. [50] showed that the RAGE is a potential drowning biomarker which was about 12 times higher in drowning group compared to the control groups. Also, the expression

of RAGE went on decreasing with time after death suggesting that it can be a suitable biomarker for calculating postmortem interval. This behaviour of RAGE expression is similar to other biomarkers used for the diagnosis of drowning deaths. It is shown by the study done by Li et al. [51] showing that other such biomarkers like 18S rRNA and microRNA levels also have a tendency to decrease gradually after death [51].

The study done by Lee et al. [36] showed that the mRNA expression of RAGE is higher in drowning cases compared to other groups. They also immunohistochemically stained the alveolar type I cells and analyzed the secretion of RAGE. Dense granular RAGE staining was observed more in drowning and hypoxia groups compared to postmortem submersion group.

Conclusion

In spite of all the recent advances as mentioned above, the diagnosis of drowning still remains a mystery in forensic science. The perfect diagnostic test still eludes the forensic specialist in case of drowning. But, recent advances in detection of drowning specific biomarkers, impedance spectroscopy, postmortem computed tomography, electrolyte estimation from various bodily fluids, etc. have shown immense potential for the creation of a drowning specific screening test. When coupled with other traditional findings, these newer diagnostic methods can help to diagnose drowning with a much greater accuracy than what is currently possible.

List of Abbreviations

HU: Hounsfield Units

MSCT: Multislice Computed Tomography

RAGE: Receptor for Advanced Glycation End Products

SP: Surfactant-specific proteins

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