Autopsy relevance determining hemochromatosis

Case report

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Abstract

Rationale: Hemochromatosis is a disorder associated with an abnormal accumulation of iron leading to toxic organ damage. Clinical symptoms develop during a long period of time, thus, determining accidental or late diagnosis, usually when complications are evident.

Patient concerns: A 53-year-old man was brought to the emergency unit with symptoms of hypovolemic shock without any apparent cause, which ultimately led to multiple organ failure, severe metabolic acidosis.

Diagnoses: The final diagnosis of hemochromatosis was determined after the autopsy.

Interventions: Abnormal findings included a black-grayish pancreas, without any surrounding tissue reaction, and a dilated congestive cardiomyopathy. Histological findings revealed significant hemosiderin deposits in the internal organs, which were more distinct in the pancreas, liver, and kidneys.

Outcomes: Patient death in less than 12 hours.

Lessons: The necessity of a genetic examination after the autopsy, regarding this case was undeniable, especially focusing on the first-degree relatives, helping to diagnose and prescribe an adequate and early treatment.

Abbreviations: ALT = alanine aminotransferase, APTT = Partial thromboplastin time, AST = aspartate aminotransferase, BMI = body mass index, GCS = Glasgow coma scale, H = hemochromatosis, INR = international normalized ratio, PT = prothrombin time, SPA = Stago protrombin assay, WBC = white blood cells.

Keywords: forensic medicine, hemochromatosis, iron storage disease, pathology

1. Introduction

Primary hemochromatosis (H) is an autosomal recessive genetic disease related to the HFE gene. Multiple studies have shown that most of the patients who had phenotypically expressed hemochromatosis had C282Y (80%) and H63D gene mutations.[1–3]

Secondary hemochromatosis is usually caused by the disorders of erythropoiesis and multiple blood transfusions. Macrophages damage transfused erythrocytes, releasing iron from hemoglobin, causing its accumulation in the body.[4,5]

Hereditary hemochromatosis is the most common inherited liver disease among the white population and one of the most common autosomal recessive genetic disorders. The prevalence of hereditary hemochromatosis is high all over the world, especially in the Northern Europe, with 1 out of 220 to 250 having the disorder.[6–7]

Despite the 30% discrepancy between clinical and pathological diagnoses in Europe and the decreasing number of performed autopsies,[8–13] cases of clinically unsuspected conditions, which are revealed after postmortem examination, have been published after various studies have been completed.[14–16] Since hemochromatosis is difficult to diagnose due to the rapid internal organ dysfunction development, autopsy remains one of the main rare condition identifying methods.

2. Materials and methods

This case report was approved by the Ethics Committee of Republican Vilnius University hospital. The patient signed the informed consents. An analysis was performed, in order to evaluate the data from the State Forensic Medicine Service, focusing on full forensic pathology autopsies between 2010 and 2016. In 2010, the number of performed autopsies was 7395, accounting for 17.3% of all deaths that year. In 2016, the number of performed autopsies was 5873, accounting for 14.3% of all deaths that year. During this period, there was only one case of hemochromatosis in Lithuania diagnosed after the postmortem examination. Toxicology tests were performed. Histological findings were analyzed using Perls’ Prussian Blue reaction, in order to detect iron accumulation in tissues. A descriptive method was used, while reviewing literature focusing on hemochromatosis.

The Perls’ Prussian Blue reaction consists of several stages. Firstly, the sections are plunged into distilled water. Secondly, the mixture of ferrocyanide and hydrochloric acid is applied for 10 minutes, following a continuous wash in distilled water for 5 minutes. A counterstain with filtered neutral red stain is applied for 1 minute with a rinse in distilled water afterward. Finally, a
quick dehydration in absolute alcohol is being performed. If ferric salts are present, they become evident in dark blue color, nuclei—red, erythrocytes—yellow.

Technical points: a known positive control section must be used; neutral buffered formalin was used; red background staining results if the neutral red stain is applied directly from tap water, differentiation of neutral red staining is achieved during dehydration.

Reagent formula: aq hydrochloric acid (analytical reagent grade); aq potassium ferrocyanide (analytical reagent grade); neutral red stain: neutral red (CI 50040) 1 g; distilled water 100 mL; glacial acetic acid 1mL. Dissolve the dye in the distilled water, add the acid, mix and filter.

3. Case

A 53 years old male, of 171 cm height and 75 kg weight (BMI 24.6) was hospitalized in the intensive care unit of Republican Vilnius University hospital in a severe general condition, due to acute abdominal pain and episodes of hematemesis continuing for 3 days. Other clinical features included: 13 points on Glasgow coma scale (GCS), heart rate of 155 beats per minute, arterial blood pressure 100/60 mm Hg, anuria was present. The abdomen ultrasound showed hepatomegaly, and liver and kidney insufficiency, electrolyte imbalance, hipocoaulation, and metabolic acidosis (Table 1). The further disease progression was rapid and uncontrollable. After 10 minutes hospitalization, the patient’s condition became critical—intubation and lung ventilation were required. The blood pressure dropped to 70/40 mm Hg, resulting in tachycardia of 172 beats per minute. After nasogastric tube insertion, a clear slightly reddish liquid was received. Bradycardia occurred 45 minutes later, leading to unsuccessful cardiopulmonary resuscitation.

The diagnosis of poisoning by unspecified substance was formulated by the hospitals toxicologist. The cause of death remained unclear, resulting in the full autopsy being performed.

### Table 1

**Laboratory tests results at hospitalization.**

| Laboratory tests | Results       | Normal range         |
|------------------|---------------|----------------------|
| WBC              | 16.3 x 10^9/L| 4.0-10.0 x 10^9/L    |
| Hb               | 119 g/L       | 140-175 g/L          |
| K                | 5.8 mmol/L    | 3.5-5.1 mmol/L       |
| Na               | 134 mmol/L    | 135-145 mmol/L       |
| Cl               | 95.4 mmol/L   | 96-107 mmol/L        |
| AST              | 717 U/L       | 8-40 U/L             |
| ALT              | 1351 U/L      | ≤ 52 U/L             |
| Total bilirubin  | 171 µmol/L    | 5-17 µmol/L          |
| Direct bilirubin | 37.4 µmol/L   | 0-8.6 µmol/L         |
| APTT             | 216 s         | 26-45 s              |
| SPA              | 6%            | 77-120%              |
| INR              | 6.3           | 0.8-1.2              |
| Fibrinogen       | 0.3 g/L       | 1.8-3.5 g/L          |
| D-dimer          | 200,000 µg/L  | < 250 µg/L           |
| Arterial blood pH| 6.975         | 7.35-7.45            |
| Arterial blood PaCO₂| 36.4 mmHg | 35-45 mmHg           |
| Arterial blood PaO₂| 21.5 mmHg | 83-108 mmHg          |
| Arterial blood lactate| 15 mmol/L  | 0-2.4 mmol/L         |
| Creatinine       | 194 µmol/L    | 27-115 µmol/L        |
| Urea             | 134 mmol/L    | 1.8-8.3 mmol/L       |
| Glucose          | 3.5 mmol/L    | <5.6 mmol/L          |

*APTT = partial thromboplastin time, SPA = stage prothrombin assay, INR = international normalized ratio, AST = aspartate aminotransferase, ALT = alanine aminotransferase, WBC = white blood cells.

During external evaluation mechanical injuries were absent. A slight, bronze skin hyperpigmentation of the face and hands was evident.

3.1. Internal autopsy findings

During the internal examination a congestive cardiomyopathy was discovered (Table 2). Further pathological findings included atherosclerotic plaques of the coronary arteries with a 50% stenosis of the lumen, myocardial fibrosis, black-greyish, rigid pancreas without any surrounding tissues reaction (Fig. 1), lung, and brain edema. The liver, kidneys, and bowel demonstrated no macroscopically evident pathology.

The toxicology tests were completed and excluded clinically suspected poisoning.

Additional histological findings showed significant hemosiderin deposits in the cardiomyocytes (Fig. 2A), pancreas acinar cells (Fig. 2B), hepatocytes (Fig. 2C), and renal tubular epithelium (Fig. 2D).
The diagnosis of the main disease of undetermined hemochromatosis was exposed relying on the postmortem examination results. The complications included congestive cardiomyopathy, metabolic acidosis, and multiple organ dysfunction syndrome. The first-degree relatives of the deceased have been informed about the nature of the disease, inheritance aspects, and the necessity of a genetic evaluation.

4. Diagnostics

4.1. Laboratory diagnostics

The disease is often diagnosed accidentally—during a prophylactic examination, when elevated blood iron level is detected. Serum transferrin saturation and serum ferritin are the 2 key indicator tests detecting iron overload. Serum transferrin saturation is meant to measure the amount of iron bound to a protein (transferrin) which carries iron in the blood. Transferrin saturation values greater than 45% are considered being high. Serological iron markers are widely available and informative, when no symptomatology is evident.[2,3,6]

4.2. Genetic examination

Detection of hemochromatosis-associated C282Y and H63D mutations is conducted to confirm the diagnosis.[2,3,6,7] Genetic evaluation is recommended if serum ferritin or serum transferrin saturation is abnormal. The American Association for the Study of Liver Diseases suggests a checkup for the selected patients, whose first-degree relatives suffered from hereditary hemochromatosis, associated with HFE gene mutation, including laboratory and genetic tests, in order to diagnose and apply an early treatment of the condition.[6]

4.3. Liver and skin biopsy

The liver biopsy is recommended for homozygotes with a clinically evident liver disease, when serum ferritin is greater than 1000 ng/mL, and particularly for those older than 40 years, with other risk factors causing liver disease. Liver biopsy should also be considered as compound of C282Y gene mutation associated heterozygotes with elevated TS, particularly those who have had abnormal liver enzyme levels or clinical evidence of liver disease.[2,6]

5. Discussion

Hereditary hemochromatosis is usually asymptomatic until the middle age is reached. Hemochromatosis becomes apparent after the age of 40 in men and after the age of 50 in women. This peculiarity can be explained by regarding menstruations as a physiological blood loss, which increases the rate of iron removal from the body. Hemochromatosis can be characterized by a classic triad: liver cirrhosis, diabetes mellitus, and skin bronzing or hyperpigmentation.[5]

5.1. Liver disease

Iron accumulates in the hepatic cells, which leads to hepatomegaly, fibrosis, and cirrhosis. Hepatocellular carcinoma along with cirrhosis could be a complication of hereditary hemochromatosis.[4,17,18]

5.2. Diabetes mellitus

Iron overload is a risk factor for diabetes mellitus development, which occurs in 13–22% of people who have hereditary hemochromatosis.[19]

5.3. Heart failure

The presence of free iron in biological systems can lead to the rapid formation of damaging reactive oxygen metabolites. These metabolites can produce DNA cleavage, impair protein synthesis, and impair cell integrity and cell proliferation, leading to cardiomyocyte necrosis.[20,21,22]
5.4. Osteoporosis
Around 25%–50% of patients are diagnosed with osteoporosis. It usually emerges due to the damage of other organs, such as hypogonadism and advanced liver disease.[23]

5.5. Skin bronzing or hyperpigmentation
A combination of iron deposits and melanin causes the skin to become bronzed or hyperpigmented, which is typical during the late stages of the disease for 90% of patients.[24]

The most common discrepancies between clinical and forensic diagnoses happen due to the clinical diagnosis being ultimately determined false, which may be influenced by several factors. The objective factors include: the short-term stay in the hospital, the severity of the patients’ condition. The subjective factors are: an inadequate anamnesis data evaluation and patient examination, an incorrect assessment of clinical data, and the inaccurate evaluation of the test results, a wrong consultant’s conclusion, and an incorrect formulation of the final diagnosis.

6. Conclusion
A diagnosis of hemochromatosis must be suspected after finding a pathological, black-greyish pancreas during an autopsy. A histological evaluation using Pearls Prussian blue staining must be performed. The necessity of a genetic examination is undeniable, when a hemochromatosis positive diagnosis is confirmed, especially focusing on the first-degree relatives, helping to diagnose and prescribe an adequate and early treatment.

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