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Review Article

Recent Update in Diagnostic Evaluation of Hereditary Hemochromatosis

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Abstract

Hereditary hemochromatosis (also called bronze diabetes) is a common autosomal recessive disorder in the Caucasian population characterized by high levels of iron accumulation due to increased dietary absorption despite a normal intake. Most cases are seen in homozygotes with a mutation of the hemochromatosis gene (HFE) protein, the common mutation being C282Y. The excess iron is deposited in many tissues as hemosiderin leading to multiple organ damage.

Most cases of hereditary hemochromatosis are asymptomatic or manifest with nonspecific symptoms in adulthood, usually between 30 to 50 years of age. They are often discovered incidentally when abnormal iron indices are noted as part of routine chemistry screening for other conditions or during screening when a family member or relative is diagnosed with hemochromatosis.

Initial testing should include testing for serum ferritin and the serum transferrin saturation, which are usually increased. Further testing is considered if the ferritin levels exceed 200 µg/L (females) or 300 µg/L (males or postmenopausal females) or if the transferrin saturation (TSAT) is greater than 45%.

Total iron-binding capacity (TIBC) values > 450 mcg/dL or > 80.55 mmol/L can be helpful in diagnosing pathological iron accumulation. The negative predictive value is 97% for iron overload with normal serum ferritin and TSAT < 45%. Hyperferritinemia alone can be a rather non-specific finding, and most people with high ferritin levels need not suffer from hemochromatosis since it is often elevated in the setting of inflammation or malignancy. A persistently increased TSAT level > 45% is a more reliable indicator of hemochromatosis. Other findings that would elicit suspicion of hemochromatosis include imaging evidence of iron overload in the liver on MRI or iron deposits in hepatocytes on a liver biopsy.

Absence of acquired risk factors for hepcidin deficiency like alcohol abuse or end-stage liver disease favor the possibility of hereditary hemochromatosis. Secondary causes of iron overload must be excluded.

Since there is no cure, it is important to detect this condition early when the patient has not developed features related to cirrhosis or irreversible tissue damage. This article outlines the approach to patients with hereditary hemochromatosis and reviews the role of various laboratory tests and investigations in evaluating such cases.

Keywords: *Hereditary Hemochromatosis; Total Iron-Binding Capacity (TIBC); Transferrin Saturation (TSAT); Hemochromatosis Gene (HFE)*

Introduction

Hereditary hemochromatosis patients are often asymptomatic. The symptoms usually manifest in adulthood, usually between 30 to 50 years of age, and can be non-specific in the early stages. Most cases are discovered incidentally when abnormal iron indices are noted as part of routine chemistry screening for other conditions or during screening when a family member or relative is diagnosed with hemochromatosis. Since there is no cure, it is important to detect this condition early when the patient has not developed features related to cirrhosis or irreversible tissue damage. This article outlines the approach to patients with hereditary hemochromatosis and reviews the role of various laboratory tests and investigations in evaluating such cases.

Etiology

First described as the syndrome of bronze diabetes and pigmentary cirrhosis ('diabète bronze´ et cirrhose pigmentaire') in 1871 [1], it is believed that the term Hemochromatosis was coined by von Recklinghausen in 1889. In 1977, Marcel Simon., *et al*. established the genetic basis for this condition by reporting the association between the major histocompatibility complex and the hemochromatosis gene on 6p chromosome [2].

The C282Y variant was initially described as the mutation associated with the HFE gene, but later on several other non-HFE genes including variants of the hepcidin (HAMP) [3,4], Hemojuvelin (HJV) [5], Ferroportin/solute carrier family 40 member 1 (SLC40A1) [6] and second receptor for transferrin (TFR2) [7] genes were identified as being responsible in a smaller number of cases.

Evaluation

The serum ferritin concentration and the serum transferrin saturation are increased. Further testing needs to be considered if the ferritin levels exceed 200 µg/L (females) or 300 µg/L (males or postmenopausal females) or if the transferrin saturation (TSAT) is greater than 45% [1].

Total iron-binding capacity (TIBC) values > 450 mcg/dL or > 80.55 mmol/L can be helpful in diagnosing pathological iron accumulation [8]. The negative predictive value is 97% for iron overload with normal serum ferritin and TSAT < 45% [9]. Hyperferritinemia alone can be a rather non-specific finding, and most people with high ferritin levels need not suffer from hemochromatosis, since it is often elevated in the setting of inflammation or malignancy. A persistently increased TSAT level > 45% is a more reliable indicator of hemochromatosis. Other findings that would elicit suspicion of hemochromatosis include imaging evidence of iron overload in the liver on MRI or iron deposits in hepatocytes on a liver biopsy.

Absence of acquired risk factors for hepcidin deficiency like alcohol abuse or end-stage liver disease favor the possibility of hereditary hemochromatosis. Secondary causes of iron overload must be excluded, these include, iatrogenic iron overload (regular transfusions), disorders associated with ineffective erythropoiesis (thalassemia and other hemolytic anemias, myelodysplastic syndromes), liver dysfunction or disease that reduces hepcidin production, chronic renal failure, adult-onset Still's disease, hemophagocytic lymphohistiocytosis and hereditary hyperferritinemia.

Genetic testing

The next step would be genetic testing for mutations.

Hereditary hemochromatosis is classified into four types [10]:

1. Type 1 (Classic, HFE-related)
2. Type 2 (Juvenile Hemochromatosis)
    a. 2a - HJV related
    b. 2b - HAMP related)

3. Type 3 (TFR2)
4. Type 4 (Ferroportin).

HFE related mutations involve the C282Y, H63D and S65C, and together these account for over 90% of the cases, of which the majority of cases are due to C282Y homozygosity. The others are much less common and include mutations involving HAMP, HJV, SLC40A1 and TRF2.

HFE, HFE2, HAMP and TFR2 mutations cause severe reduction in hepcidin synthesis which leads to increased intestinal iron absorption and tissue iron overload, the severity of hepcidin down-regulation determines the severity of iron overload and clinical complications. Ferroportin disease is a result of loss-of-function mutation of SLC40A1 that impairs the iron export efficiency of Ferroportin. This leads to iron retention in reticuloendothelial cell and hyperferritinemia with normal transferrin saturation [11].

HFE genotyping is recommended as the first diagnostic step in patients with high serum ferritin and TSAT levels [12,13]. C282Y homozygosity is associated with a majority of cases with an increased risk of developing liver cirrhosis but there is a significant phenotype variation due to incomplete penetrance [14]. There is a significant positive statistical association between abdominal pain and cirrhosis in non-screening hemochromatosis probands with HFE p.C282Y homozygosity [15]. The incidence of C282Y or H63D heterozygotes is less than 5% [16].

Since mutations in HJV, HAMP, TFR2 or Ferroportin are far less common, genetic testing for non HFE hemochromatosis may be considered in cases where other causes of hyperferritinemia have been ruled out, there is family history of iron overload [17], or hepatic iron overload can be demonstrated on MRI or liver biopsy. HJV, HAMP and TFR2 mutations are usually seen in early onset disease with severe clinical manifestations and are recessive while the Ferroportin mutations are dominant [18,19].

Testing for liver involvement

Liver biopsy with histochemical, semi-quantitative assessment or chemical determination of iron content was considered the gold standard for diagnosis of hemochromatosis but demonstration of specific mutations with increased levels of Serum ferritin and Transferrin saturation (TSAT) are now the norm. It is now done in cases where the diagnosis is unclear e.g. certain types of non-HFE hemochromatosis, dysmetabolic iron overload syndrome (DIOS), non-alcoholic fatty liver disease (NAFLD) [20] as well as some types of alcoholic liver disease presenting with elevated ferritin and moderate iron overload. It can also be useful in assessing the degree of fibrosis and also for detecting hepatocellular carcinoma [21].

On a liver biopsy, the golden yellow hemosiderin granules are typically seen as cytoplasmic deposits within hepatocytes initially in the periportal region (Image 1) and later on within the rest of the lobule including the Kupffer cells and the biliary epithelium. These granules will appear blue with the Prussian blue stain (Image 2). In the early stages, the parenchymal architecture is preserved but eventually will be distorted with the onset of cirrhosis.

Non-invasive techniques like MRI are now preferred to assess the liver iron concentration in favor of the older invasive biopsy [22,23]. The use of MRI for noninvasive hepatic iron quantification can also improve the diagnostic yield of next-generation sequencing (NGS) in patients with hyperferritinemia [24].

Other non-invasive techniques for detecting liver fibrosis in patients with hemochromatosis include Aspartate aminotransferase-to-platelet ratio index (APRI), Fibrosis-4 Index (Fib4) Hepascore and Hepatic transient elastography (FibroScan) [25-27]:

- APRI: [(AST (U/L)/upper limit of normal) × 100/platelet count (109/L)]
- Fib4: [age (years) × AST (U/L)] / [platelet count (109/L) × ALT (U/L) ½]
- Hepascore: y/ (1 + y), where y = exp [(-4.185818 – 0.0249 × age + 0.7464 × sex (male = 1, female = 0) + 1.0039 × α-2-macroglobulin (g/L) + 0.0302 × hyaluronic acid (μg/L) + 0.0691 × bilirubin (μmol/L) – 0.0012 × GGT (U/L)].

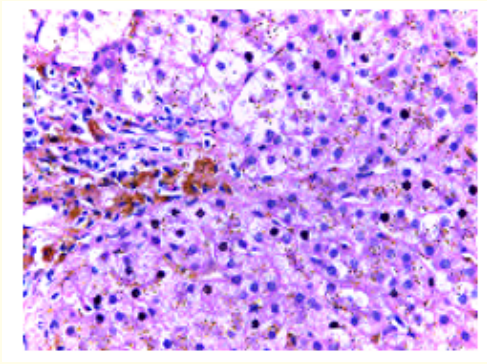

Image 1: *Micrograph of hemochromatosis liver showing hepatocytes with coarse golden yellow granules of hemosiderin within the cytoplasm. These granules stain with Prussian blue stain. (By Calicut Medical College - Department of Pathology, Calicut Medical College, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=44955377).*

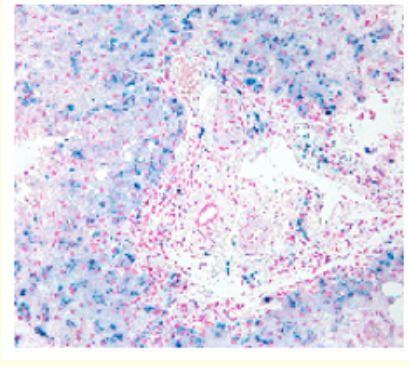

Image 2: *High magnification micrograph of hemosiderosis. Liver biopsy. Iron stain (Prussian blue). (By Nephron - Own work, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=7765904).*

The APRI cut-off value > 0.44 and Fib4 cut-off value > 1.1 have shown good diagnostic utility in correctly identifying liver biopsy diagnosed advanced hepatic fibrosis in 85% and 80% of cases, respectively [28]. Hepascore assesses clinical variables of sex and age and combines these with blood-based markers including bilirubin, gamma-glutamyl transferase (GGT), hyaluronic acid and alpha2-macroglobulin to detect hepatic fibrosis. Transient elastography (TE) uses ultrasound to and can be done in an outpatient setting. Both Hepascore and TE however, seem to be more reliable in cases where the serum ferritin levels exceed 1000 µg/L [29].

Testing for cardiac involvement

The iron deposits accumulate within the cardiac myocytes beginning in the epicardium and then moving towards the endocardium. Initially there is hypertrophy, leading on to diastolic dysfunction, dilated cardiomyopathy, systolic dysfunction and arrhythmias. It has been suggested that assessing serum non-transferrin bound Iron (NTBI) levels might be useful to initiate early treatment to avoid damage to organs including the heart but it is not an easy test to standardize [30].

Electrocardiography changes are usually non-specific and recognized late in the course of the disease but Standard echocardiography can be particularly useful even in the early stages to reveal cardiac abnormalities. The two-dimensional Speckle Tracking Echocardiography (2D STE) and three-Dimensional (3D) Real-Time Echocardiography are novel techniques that seem to be more precise and distinctive in detecting subtle abnormalities.

Cardiac magnetic resonance (CMR): The value of this technique is indisputable in the diagnosis of cardiac abnormalities caused by HH, a reduction of T2 relaxation < 20 ms is considered a symptom of iron overload, but the cost and availability have been limiting factors in everyday practice [31].

Cardiac biopsy is risky and is not routinely performed. It may be indicated in cases where the diagnosis is not clear and other causes of heart failure or infiltrative diseases need to be ruled out [8].

Testing for pancreatic involvement

Fasting glucose levels or Glucose tolerance test will confirm a diagnosis of Diabetes. Iron deposition in the pancreas can result in hyperglycemia and HH should be suspected if there is skin hyperpigmentation, joint pain, hypogonadism or features of liver disease [32]. The pathogenesis of diabetes in HH is considered multifactorial, both insulin deficiency and resistance may contribute. Iron overload can impair insulin secretion and glucose tolerance early in hereditary hemochromatosis, before cirrhosis occurs [33].

Iron uptake into the beta cells leads to impaired insulin synthesis and release and the liver fibrosis leads to high levels of circulating insulin and insulin resistance. Increased type 2 diabetes risk in HFE hemochromatosis is associated with one or more factors, including abnormal iron homeostasis and iron overload, decreased insulin secretion, cirrhosis, diabetes in first-degree relatives, increased body mass index, insulin resistance, and metabolic syndrome [34].

Hemochromatosis can also less commonly, result in chronic pancreatitis and MRI can be effective in assessing iron deposition in the pancreas [35].

Testing hormone levels to evaluate hypogonadism

After diabetes, hypogonadism is the second most common endocrine abnormality in HH, frequency ranging from 10 to 100% [36]. Patients can present with erectile dysfunction, decreased libido, decrease of androgen-dependent hair pattern, testicular atrophy and azoospermia. This could be due to testicular damage caused by iron deposition but currently the favored theory is pituitary involvement leading to disturbance of the gonadotropic axis. Iron deposits in the gonadotropes of the anterior pituitary result in a decreased production of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) [37].

Patients diagnosed with hereditary hemochromatosis and suffering from infertility or other reproductive disorders should undergo a hormonal check-up including Testosterone, FSH, LH and a Gonadotropin Releasing Hormone (GnRH) test. A brain Magnetic Resonance Imaging (MRI) would help detect pituitary iron deposits.

In non-HFE forms of HH, iron deposition starts early and is more severe and seems to deposit in the pituitary, especially the gonadotropes and less commonly the other lineages [38]. In such cases patients should probably be tested for endocrine abnormalities even in the absence of complaints [39]. There is suggestion of a hormonal check-up once Ferritin is ≥ 300 μg/L, regardless of clinical manifestations in a male patient with hereditary hemochromatosis [40].

Hypogonadism in premenopausal women is evidenced by decreased levels of luteotropic and follicle stimulating hormone, decreased libido and amenorrhea [41].

Testing for musculoskeletal involvement

Joint pain is a frequently reported ailment; it may affect up to 75% of patients even in the period before diagnosis is established. Most patients report symptoms of arthropathy involving the metacarpophalangeal (MCP), ankle, knee, hip, or proximal interphalangeal joints

and may later require joint replacement surgery [42]. Overt clinical symptoms usually appear in the fifth decade of life although they may appear even in the third decade [43].

X-ray of the joints is used to evaluate the extent of arthritis. A rheumatological scoring system, based on X-rays of hands, wrists, knees and ankles, has been validated in a cohort of hemochromatosis patients with arthritis [44]. Dual-energy X-ray absorptiometry (DXA scan) is used to determine bone density and examine for osteopenia and osteoporosis [45]. The most recently introduced tool is the trabecular bone score (TBS) obtained from the spatial grayscale analysis of DXA images. As it enables the evaluation of the bone microarchitecture, the TBS can be useful as an independent and supplementary tool for bone evaluation [46].

Haemochromatosis associated pseudogout (chondrocalcinosis) is not common, but in those with severe chondrocalcinosis, the frequency of association may justify screening for haemochromatosis especially in younger male patients [47]. Inhibition of the pyrophosphatases and the synovial iron sequestration are likely mechanisms causing damage to the articular cartilage [48].

Screening tests:

- **Screening for mutations in first degree relatives of probands:** It is important to detect asymptomatic carriers. When a person (proband) is diagnosed with HFE-hemochromatosis, the closest genetic relatives (i.e. the proband's biological parents, siblings and children) should be examined with HFE-genotyping as to whether they have inherited the mutation(s). Due to the high frequency of C282Y and H63D heterozygosity in the Danish population, it is also recommended to examine the proband's partner, when they have children. The examination of children may be postponed until it is convenient for both parents and children or until the children become mature [49].
- **Population screening for HFE-hemochromatosis:** HFE-hemochromatosis meets the World Health Organization's criteria for screening [50], and a study in the Danish population reflect a very positive attitude towards screening for HFE-hemochromatosis [51]. An Australian study has demonstrated that several modalities of screening appear to be cost-effective for the society [52,53].

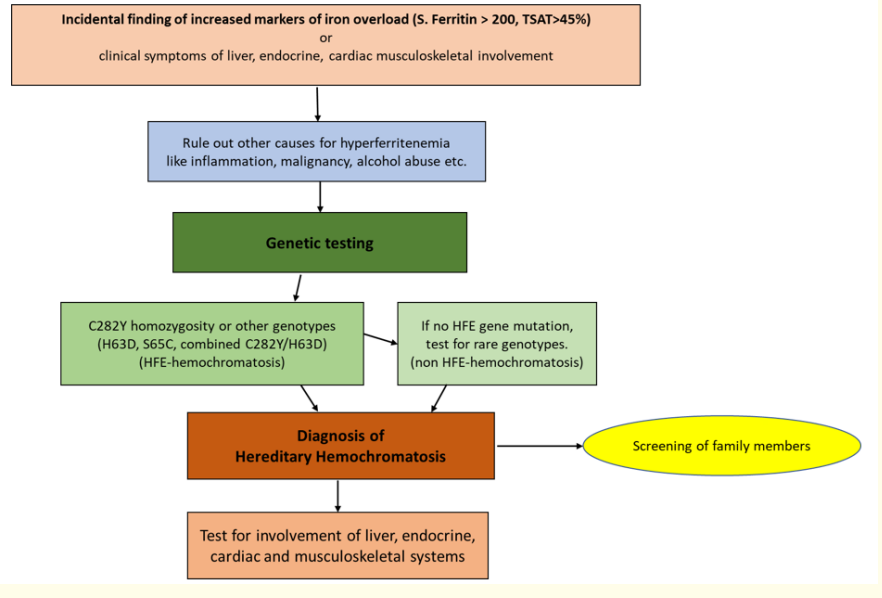

Image 3: *Approach to investigating a case of hereditary hemochromatosis (By Jeevan Divakaran).*

Conclusion

Early diagnosis is crucial to preventing irreversible multiple organ damage in cases of hereditary hemochromatosis and improving patient outcomes. This requires appropriate laboratory evaluation of suspected cases and screening of family members. An interprofessional effort from health care providers including awareness of this condition and the current testing modalities available by the patient's primary care provider will aid in preventing progressive organ injury associated with untreated cases. Referral to consultants like gastroenterologists, rheumatologists, endocrinologists, cardiologists, and hepatologists for further management will lead to an improved prognosis.

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