Dehydrated hereditary stomatocytosis masquerading as primary haemochromatosis: a diagnostic challenge

To the Editor,
Hereditary xerocytosis or dehydrated hereditary stomatocytosis (DHSt; OMIM #194380) is an autosomal dominant non-immune congenital haemolytic disorder with a prevalence of approximately one in 50,000. Although DHSt was a recognised red cell disorder, the underlying genetic defect in PIEZO1 was first described almost simultaneously by three groups in 2012 and 2013.1-3 Clinical features are variable, characterised by compensated mild to moderate haemolysis, often with self-resolving generalised oedema or ascites in the early neonatal period. Marked iron overload is common in DHSt, despite most patients being transfusion-independent.

Laboratory diagnosis of DHSt is challenging as peripheral smear features are subtle (e.g., stomatocytes usually comprise less than 10% of all erythrocytes), and other findings, like increased mean corpuscular haemoglobin, reticulocytosis, and increased erythrocytic resistance to osmotic lysis, are relatively less specific. Definitive tests like genetic testing, electrophoresis, and measurement of erythrocyte Na+/K⁺ are often unavailable. DHSt may be misdiagnosed clinically as hereditary spherocytosis or any other non-immune haemolytic anemia due to overlapping features or complex genotypes. In older persons, it may mimic myelophytic syndromes.4-5 Accurate diagnosis is of utmost importance as splenectomy in patients with PIEZO1 defects may precipitate thromboembolic events, which occasionally can be life-threatening.6 We report a delayed diagnosis of DHSt due to a PIEZO1 gene defect in a patient who was considered to have hereditary haemochromatosis for three decades.

The gentleman first presented in 1991 at the age of 40 years for an episode of severe ‘food poisoning’ following which he developed severe anaemia and jaundice. The latter did not resolve after antimicrobial therapy. He received two units of packed erythrocytes (the first transfusion of his life) along with corticosteroids and iron supplementation during that hospital stay. The haemoglobin nadir was 60 g/L in that admission, and while records are sketchy, the possibility of a genetic aetiology was not investigated.

He was subsequently evaluated in the Hepatology Department in our institute over the next year (1992) for persisting jaundice (unconjugated hyperbilirubinaemia), bilateral knee pain, and generalised skin hyperpigmentation. A complete blood count and haemolytic anaemia work-up comprising of plasma and urine haemoglobin levels, glucose-6-phosphatase dehydrogenase (G6PD) deficiency screening, direct antiglobulin test, test for haemosiderinuria, and haemoglobin electrophoresis were performed, which were non-contributory. Upper gastrointestinal endoscopy was normal, computed tomography (CT) of the abdomen showed increased attenuation of the liver parenchyma, and a liver biopsy was performed. This showed increased iron deposits without fibrosis or inflammation, suggesting primary haemochromatosis. Family history was negative.

He was initiated on injection desferrioxamine and switched over to oral deferiprone in 2002. Ferritin levels remained over 1000 ng/mL, and phlebotomies were instituted every 4–6 months after 1998. His haemoglobin levels were 161 g/L when the phlebotomy was started. Tests for haemolytic anaemia, repeated over the years, were normal. DNA analysis of the coding regions of the HFE, HJV (HFE2), HAMP (HFE2B), TFR2, and SLC40A1 genes implicated in hereditary haemochromatosis was negative.1 Work-up for Gilbert syndrome was negative [UGT1A1 (TA) promoter repeats were 6/6]. He was lost to follow-up in 2004.

In 2016, he presented with gradually progressive massive splenomegaly (16 cm below the left costal margin), portal hypertension, grade 3 oesophageal varices and gall bladder sludge. Haemogram showed pancytopenia (haemoglobin 79 g/L, total leukocyte count 3.7 x 10⁹/L, platelet count 64 x 10⁹/L) which was attributed to hypersplenism. Hyperpigmentation, jaundice and anaemia persisted. He received a trial of erythropoietin and eltrombopag, without improvement in blood counts. Contrast-enhanced magnetic resonance imaging (MRI) revealed liver parenchymal disease. T2*-weighted MRI in 2017 showed a myocardial iron concentration of 1.15 mg/g of dry myocardium (reference range <1.16 mg/g) and liver iron concentration of 5.64 mg/g of dry liver, suggesting a light degree of deposition (normal <2 mg/g, light deposition 2–6.9 mg/g, moderate 7–15 mg/g, severe >15 mg/g). Serum ferritin level was 445 ng/mL (20–250) while percentage transferrin saturation was 51.3% (20–40).

Upper gastrointestinal endoscopy in 2016 showed portal hypertension with grade II-III oesophageal varices and mild portal hypertensive gastropathy. Endoscopic variceal ligation was done. Ultrasonography, liver stiffness measurement by fibroscan and acoustic radiation force impulse-shear-wave elastography revealed no evidence of cirrhosis.

In view of the obscure diagnosis, percutaneous liver biopsy was repeated, which revealed mild portal fibrosis and coarse haemosiderin pigment in perportal hepatocytes and in a few Kupfer cells suggestive of haemochromatosis. Quantitative iron estimation yielded a concentration of 6722 μg/g dry weight of the liver (200–2400).

The aetiology for portal hypertension and oesophageal varices could not be definitively established. Secondary haemochromatosis-related progressive hepatic fibrosis was suspected as a possible cause, but imaging studies and fibroscan did not provide conclusive evidence.

In this admission, moderate macrocytic anaemia [haemoglobin 74–95 g/L (120–140), haematocrit 24.1% (41–53), red blood cell count 2.04 x 10¹²/L (4.5–5.9), mean cell volume 115.6 fl (80–100), mean corpuscular haemoglobin 39.6 pg (26–34), mean corpuscular haemoglobin concentration 34.3% (31–37), red cell distribution width-coefficient of variation 18.3% (11.0–14.5), total leukocyte count 3.5 x 10⁹/L (4.0–11.0), platelets 90 x 10⁹/L (150–450), indirect hyperbilirubinaemia, total/conjugated bilirubin 8/1.26 mg/dL (0–1.0/0–0.3), and reticulocytosis 14% (1.0–2.7) were found. Peripheral smear showed reduced red blood cell density with predominant macrocytic normochromic red cells, moderate anisopkilocytosis, stomatocytes, reduced platelets, and occasional nucleated red blood cells (Fig. 1A–
D). Haptoglobin level was 5.83 mg/dL (36–195), and lactate dehydrogenase was 212.36 U/L (240–480). A bone marrow evaluation showed megaloblastic erythroid hyperplasia (myeloid to erythroid ratio reversed to 1:3) with adequate megakaryocytes and low bone marrow iron stores.

Haemolytic anaemia work-up was done, and common causes, including beta-thalassaemia, unstable haemoglobins, hereditary spherocytosis, G6PD deficiency, paroxysmal nocturnal haemoglobinuria (PNH) and autoimmune haemolytic anaemia, were excluded. Based on the current findings of splenomegaly, unconjugated hyperbilirubinaemia, moderate anaemia, reticulocytosis, and the few stomatocytes, targeted next-generation sequencing (NGS) analysis was performed when the patient was aged 68 years. It revealed a heterozygous missense variant NM_001142864.2(PIEZO1):c.7367G>A (p.Arg2456His) (rs587776988) in exon 51 of the piezo-type mechanosensitive ion channel component 1 (PIEZO1) gene. This gain-of-function variant was confirmed on Sanger sequencing (Fig. 1E). It has been previously reported in the literature to be pathogenic, causing DHSt/hereditary xerocytosis.1,2,8 This variant was not found in all three asymptomatic children (aged 38, 35, and 30 years) of the index case. The serum ferritin level and percentage transferrin saturation were within normal limits in all the children.

Another heterozygous missense variant NM_000463.3: c.1456T>G (p.Tyr486Asp); rs34993780 was found in the exon 5 of UGT1A1 gene. This variant is reported in ClinVar (RCV000999563.2) and OMIM (#191740.0017) to be likely pathogenic, causing Gilbert syndrome. In this patient, the synergistic effect of this heterozygous variant may have exacerbated the hyperbilirubinaemia.

Apart from these, a heterozygous missense variant NM_022436.2:c.733G>A (p.Val245Met); rs774899900 was found in exon 6 of the ABCG5 gene. This variant was classified as a variant of uncertain significance (VUS) based as per the American College of Medical Genetics and Genomics (ACMG) guidelines. The gene is implicated in sitosterolaemia, an autosomal recessive disorder characterised by stomatocytosis and macrothrombocytopenia. This position is highly conserved (minor allele frequency of T=0.000004 in gnomAD). In silico analysis revealed a probably deleterious effect. However, this heterozygous variant may have contributed to the presence of stomatocytes that are seen with heterozygosity of ABCG5/8 genes.11

DHSt/hereditary xerocytosis is a rare erythrocytic membrane transport defect. Most patients show compensated haemolysis with/without macrocytic anaemia. Stomatocytes are not as distinctive as seen in overhydrated hereditary stomatocytosis and often are <10%. Picard et al.9 reported a

![Fig. 1](image-url) (A) Red blood cells showing mild macrocytosis, few stomatocytes, reduced platelets, and nucleated red blood cells; (B) reticulocytosis; (C, D) presence of stomatocytes; (E) Sanger sequencing chromatogram showing heterozygous missense variant NM_001142864.2:c.7367G>A (p.Arg2456His) (rs587776988) in exon 51 PIEZO1 gene.

Please cite this article as: Jamwal M et al., Dehydrated hereditary stomatocytosis masquerading as primary haemochromatosis: a diagnostic challenge, Pathology, https://doi.org/10.1016/j.pathol.2023.03.016
large series (126 patients) of patients with DHSt. The mean age of diagnosis was 32±18 years in the index cases with the youngest being diagnosed prenatally and the oldest being diagnosed at the age of 88 years. The common clinical features at the time of diagnosis are non-spherocytic chronic haemoly-sis, splenomegaly, cholelithiasis, hyperbilirubinaemia, and hyperferritinaemia. Perinatal oedema was noted in 17% of all cases and post-splenectomy thrombotic events were seen in 11%. The affected family members were diagnosed early based on the genetic findings. Our patient had no history of perinatal oedema or pseudohyperkalaemia, and the phenotype was similar to Zarychanski et al. and Andolfo et al. The mean bilirubin levels reported by Picard et al. were 2.5±1.4 mg/dL; however, our patient had higher levels of bilirubin (8 mg/dL), mostly unconjugated, which may be contributed by the co-inheriting heterozygous missense variant [c.1456T>G (p.Tyr486Asp)] in the UGT1A1 gene. DHSt can also mimic acquired non-haemolytic disorders, including myelodysplastic syndromes. In our case, we had the history not been long, the cytopenias, iron overload, and age would have also indicated the myelodysplastic syndromes.

The p.Arg2456His is one of the recurrent variants causing DHSt/hereditary xero-cytosis. This genetic variant was shown by Andolfo et al. to result in increased cation transport across the PIEZO1 protein channel. This variant has not been previously reported in India and due to compensated haemolysis or mild phenotypes, stomatocytosis may be an underdiagnosed condition. Our patient was diagnosed with hereditary haemochromatosis based on siderosis in the liver and was treated for haemochromatosis (iron chelation and phlebotomy) for approximately 30 years. It is now well known that DHSt patients have secondary iron overload; however, the pathogenesis for having severe iron overload in these cases is not well understood. Hereditary haemochromatosis due to genetic defects in HFE, HJV (HFE2), HAMP (HFE2B), TFR2, and SLC40A1 are rare in India. Based on this case, it is recommended to test all patients with unexplained iron overload (especially those with reticulocytosis) by NGS for a hereditary chronic haemolytic disorder. The advent of NGS-based genetic diagnosis has led to rapid and accurate diagnosis of such complex cases where phenotypes masquerade for another related disease.

Conflicts of interest and sources of funding: The funding was sanctioned to Dr Reena Das from Department of Biotechnology, Ministry of Science and Technology, New Delhi, India vide no. BT/PR12682/MED/12/689/2015. The authors state that there are no conflicts of interest to disclose.

Manu Jamwal1, Namrata Singh1, Prashant Sharma1, Ajay Duseja1, Deepesh P. Lad1, Pankaj Malhotra1, Reena Das1

1Department of Hematology, Postgraduate Institute of Medical Education and Research, Chandigarh, India
2Department of Hematology, Postgraduate Institute of Medical Education and Research, Chandigarh, India
3Department of Clinical Hematology and Medical Oncology, Postgraduate Institute of Medical Education and Research, Chandigarh, India

Contact Dr Reena Das.
E-mail: reenadaspgi@hotmail.com

Please cite this article as: Jamwal M et al., Dehydrated hereditary stomatocytosis masquerading as primary haemochromatosis: a diagnostic challenge, Pathology, https://doi.org/10.1016/j.pathol.2023.03.016


DOI: https://doi.org/10.1016/j.pathol.2023.03.016