Case report:

Saudi Child with a Novel Mutation Causing Hereditary Spherocytosis, a Case Report

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Abstract

Spherocytosis is a descriptive terminology of red blood cells, which has a spherical shape rather than the normal biconcave shape of the normal red blood cells.

Hereditary spherocytosis (HS) is an inherited blood disorder, which has large heterogenic presentation with both autosomal dominant inheritance and less frequently in an autosomal recessive mode of inheritance.

Saudi Arabia is known to have high consanguineous marriage, which makes the population on a higher risk of developing different inherited disorders including HS.

We are reporting a novel pathological mutation in a child with HS which caused him to have hemolytic anemia since the age of 2 years. To our best knowledge, this is the first time this new mutation is reported in the medical literatures.

Keywords:
Hereditary Spherocytosis, Mutation, Hemolytic Anemia, Consanguinity, Saudi Arabia.

الملخص

طفل سعودي يعاني من طفرة جينية جديدة تسببت في تكور كريات الدم الحمراء الوراثي .

تقرير حالة طفل.

مرض تكور كريات الدم الحمراء هو مصطلح وصفي لخلايا الدم الحمراء التي لها شكل كروي بدلاً من شكل ثنائي الموجة لخلايا الدم الحمراء الطبيعية.

تكور كريات الدم الحمراء (HS) هي اضطراب دموي وراثي ، ولديه اعراض كثيرة غير متجانسة حيث يظهر كحالة وراثية متنحية وبشكل اقل شيوعًا كحالة وراثية جسدية سائدة التوريث مما يسبب تكسر انحلالي للدم.

من المعروف أن المملكة العربية السعودية لديها نسب عالية من الزيجات بين الأقارب ، مما يجعل الاجيال كثر عرضة لخطر الإصابة باضطرابات وراثية بما في ذلك تكور كريات الدم الحمراء الوراثي HS.

في تقرير هذه الحالة نسجل طفرة جينية مرضية جديدة في طفل سعودي مصاب بتكور كريات الدم الحمراء مما تسبب في إصابته بفقر دم انحلالي منذ عمر العامين. على حد علمنا هذه هي المرة الأولى التي يتم الإبلاغ عن هذه الطفرة الجينية في المجلات الطبية.

الكلمات الدالة

تكور كريات الدم الحمراء ، طفرة ، فقر الدم الانحلالي ، الأقارب ، المملكة العربية السعودية.

Introduction:

Hereditary spherocytosis (HS) are a group of inherited disorders in which Red blood cell (RBC) membrane is affected and partially lost, causing a non-immune hemolytic anemia of different severity due to decrease life span attributed to the loss of the ability of deformability found in the normal RBCs.

It was since 1871 when spherocytosis was first reported, then the genetic nature of the disease was suggested by Wilson in 1980, after which it was described subsequently with time in more detail. [1, 2]

HS is more common in certain ethnicities like in North Europe [2]. In the United States of America, the incidence of HS is estimated to be 1 in 5,000 where most patients have an autosomal dominant inheritance though, around 25% of cases have no obvious family history of the disease, which is attributed to new mutations in affected persons[3,4]. Osmotic fragility testing of suspected patients are helpful but the advanced techniques are more specific and sensitive in diagnosing cases and finding the genetic mutations causing the abnormalities.

Material: A Case Report

Our patient is a 12-year-old boy who presented to the department of Pediatrics' hematology clinic in Madinah Saudi Germany Hospital in January 2018 with history of recurrent easy fatigability, pallor, and attacks of jaundice along with dark discoloration of urine caused by urobilinogen since the age of 2 years.

The family sought medical advice several times, where he was labeled as a case of iron deficiency anemia.

He was treated with oral iron in different occasions but with no significant improvement in his hemoglobin. He received blood transfusion twice in the past for low hemoglobin reaching 7 g/dl and symptoms of anemia, but without definitive diagnosis. Folic acid supplement was started two years ago, after which he started to feel some improvement.

His mother gave a history of iron deficiency anemia, but she never received blood transfusion, and she denied any history of a blood disorders in the family nor history of splenectomy. Parents are second-degree cousins having another son and daughter who are normal.

His examination revealed that he looked well with normal growth parameters in the 25th centile for weight and height, stable vital signs and with no obvious dysmorphic features. He was active, pale with mild jaundice affecting mainly his sclera. He had no lymphadenopathy, his spleen was palpable 3 cm below the costal margin.

His liver border was just palpable below the right costal margin with a liver span of 10 cm. His heart examination showed normal heart sounds with a soft hemic murmur mainly heard over the aortic area. He had normal skin examination with no petechial rash or bruises. The central nervous system examination was grossly intact.His laboratory investigations included a complete blood count CBC and differential (Table 1).

Table 1: CBC, Differential

Tests	Results	Normal ranges*
WBC	5.3	4-15.5 x 10^3 µ/L
HGB	98	12-16 g/L
RBC	3.6	4.3-5.7 mil/ µL
Hct	30	38-80 %
Platelets	230	150-400 10^3/ µL
MCV	64	68-79 Fl
MCH	30	19-28 Pg
MCHC	42	30-38 g/dL
RDW	15.5	11-15%
Retics	7.5	0.5-1.5%
Absolute Retics Count	309	10-110x10^9/L
Neutrophils	48.6	40-74%
Lymphocytes	34.8	14-46%

* From El-Hazmi, Mohsen AF, and Arjumand S. Warsy.
"Normal reference values for hematological parameters, red cell indices, HB A2 and HB F from early childhood through adolescence in Saudis." Annals of Saudi Medicine 21.3-4 (2001): 165-169.

His peripheral blood smear showed numerous spherocytes with no abnormalities in other cell lines while his Coombs test was negative and the electrophoresis showed Hgb A 97.8%, Hgb A2 2.2% (normal up to 2.5%).

His liver function tests are shown in (Table 2) while his Urea, Creatinine, Electrolytes and blood gases were all within normal limits.

Table 2: Liver function tests*

Test	Results	Normal range
Aspartate aminotransferase (AST)	25	10-43 U/L
Aminotransferase (ALT)	30	10-100 U/L
Alkaline phosphatase	494	24-147 U/L
Bilirubin total	33.7	1.71-20.5 µmol/L

Test	Results	Normal range
Bilirubin direct	10.8	0-5.1 µmol/L

*From Thapa, B. R., and Anuj Walia. "Liver function tests and their interpretation." The Indian Journal of Pediatrics 74.7 (2007): 663-671.

Osmotic fragility test could not be done in our lab nor eosin-5-maleimide binding test, acidified glycerol lysis test or sodium dodecyl sulfate-polyacrylamide gel electrophoresis. His coagulation profile while his urine analysis showed moderate urobilinogen.

Ultrasound abdomen showed splenomegaly of 11.4 cm and hepatomegaly of 11.9 cm, with gallbladder having mild thick wall and few gallbladder stones not obstructing the common bile duct and normal looking other organs.

Management

Our patient is managed with a folic acid supplement with regular follow up in hematology clinic to monitor his hemoglobin levels along with liver functions and his family were referred to genetic counselling Screening for mother was normal, but we could not bring the father to be tested.

Surgical splenectomy following the HS guidelines published in 2004 was deferred as long as our patient is classified in mild to moderate group and that he remained stable [5].

Molecular genetic analysis for spherocytosis

Using enriched sequence capture technology of Roche/NimbleGen next-generation sequencing (NGS) sequenced on an Iliumina system; the coding exons of known spherocytosis genes was analyzed and revealed the following results (figure 1)

Figure 1: Variant identified:



Interpretations:

The sample was sent to Becenti laboratories in Germany. Using Next-generation sequencing (NGS) analysis a heterozygous variant identified the in c.281G>A p. (Trp94*) in the SLC4A1 gene, which leads to a pre-mature stop code and subsequent mRNA degradation (nonsense-mediated decay) or truncation of the protein.

To the best of our knowledge, the variant has not been described in the literature so far in Human Gene Mutation Database (HGMD 2019.2). Allele frequency of this variant was not documented in the general population as it is not been documented in genome aggregation data base (gnomAD) and this is the first time we have detected in our internal database considering the available information the variant is classified as pathogenic[6].

The pathogenic variance in the SLC4A1 gene (OMIM 109270) cause autosomal dominant spherocytosis type 4 and a segregation analysis of the identified variant in the parents to evaluate a possible de novo occurrence was advised using targeted molecular genetic testing of the family members of the patient. There is a 50% probability that the patient will transmit the SLC4A1 variant c.281G>A p. (Trp94*) to any of his offspring.

Methods:

DNA genomic was fragmented, both the end of the coding exons of the analyzed genes, along with the corresponding exon-intron boundaries, which were enriched using the Roche/NimbleGen sequence capture technology (SeqCap MedExome Library), it was amplified and sequenced at the same time by Illumina technology NGS using an Ilumina system. Requested gene panel was extracted from the whole-exome sequencing (WES) data. Target regions were sequenced covering almost 143-fold handling more than 99% of the regions of interest which obtained a 15-fold coverage. Using bioinformatics analysis tools as well as JSI Medical Systems software the data of NGS analysis was conducted. Against external and internal databases the identified variants and indels were filtered depending on their allele frequency with focusing on rare variants with a minor allele frequency (MAF) of 1% or less. In silico analysis of identified non-synonymous variants was performed using bioinformatics prediction programs like Mutation Taster, Polyphen-2, Mutation Assessor, FATHMM, etc. in silico analysis of spice site effects was performed by bioinformatics programs like Fruitfly, NetGene2, Human Splicing Finder, Mutation Taster, Splice view and ESE-Finder. These results should be interpreted having in mind the clinical findings, family history, and other laboratory investigations data as it is believed that the prediction programs are not equivalent to functional proof. The American College of Medical Genetics and Genomics ACMG guidelines was used for classification of variants [7].

A change of pathogenicity classifications over time cannot be excluded. Variants

class fed as (likely) benign are not reported. An insufficiently covered of the analyzed genes (A) were completed using polymerase chain reaction (PCR) amplification then was followed by conventional Sanger sequencing.

Putatively pathogenic differences between the wild type sequence (human reference genome according to UCSC Genome Browser: hg19, GRCh37) and the patient's sequence mentioned and interpreted in our case report were assessed using an in-house established quality score by Bioscientia laboratories. Variants reported and not passing the quality threshold were verified using PCR amplification then by conventional Sanger sequencing.

The sample identity was ensured by internal quality management procedures.

It is of importance to know the limitations of the NGS method as the coverage of WES data is partially far below target panel sequencing of genes for a distinct indication. In comparison, the evaluated sensitivity of WES versus panel sequencing is >99.5% (in-house validation data of Bioscientia) and that the pathogenic variants in regions which is insufficiently covered and other rare alterations in parts of the genes like in regulatory regions, low-level mosaics, or deep intronic splice variants cannot be excluded with this analysis. Furthermore, copy-number variations (e.g., deletions/duplications), complex structural rearrangements (inversions, translocations), and repeat expansions can be missed. Coding regions for which highly-homologous sequences exist in the genome are partially difficult to interpret.

This test was developed and its performance characteristics determined by Bioscientia, Ingelheim, Germany and it is not approved by the US Food and Drug Administration.

Discussion:

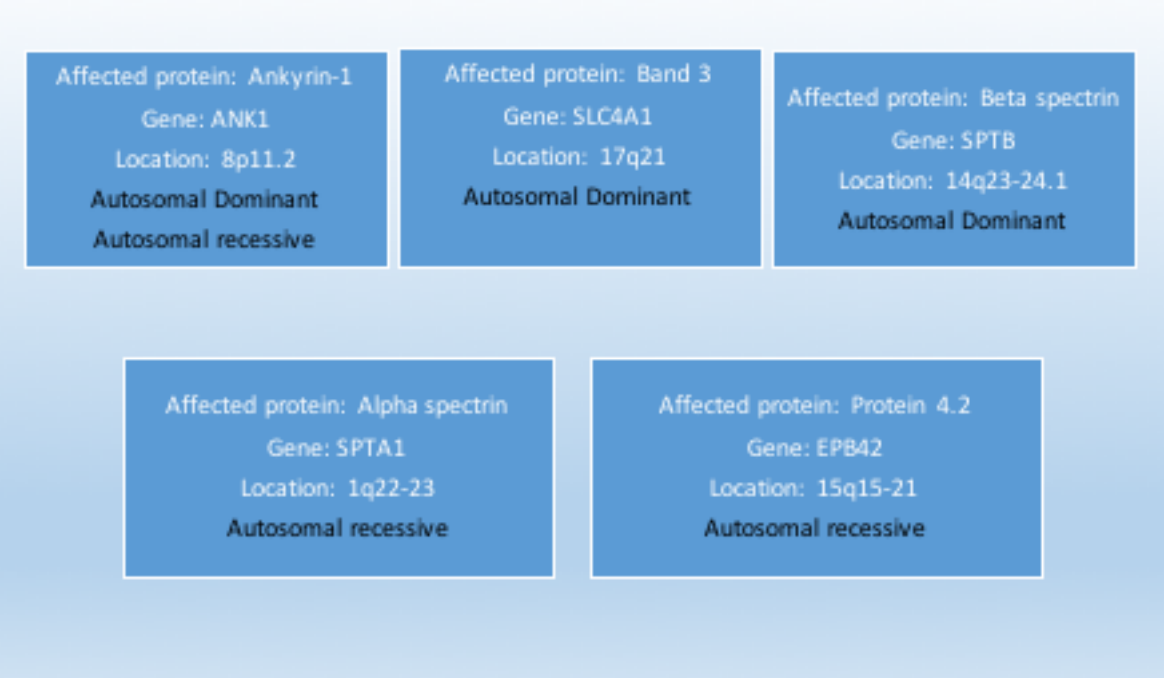
We lack population-based studies in Saudi Arabia in order to determine the incidence of HS in our population and to determine the most common types of mutations of those abnormalities.

Most of the published data are hospital-based and of a small number of patients [8].It is reported based on a small number of patients that health normal Saudis are not different from healthy Americans in regards to the RBCs membrane proteins, while on the other hand, they documented differences in the electrophoretic patterns of Saudi HS patients compared to deferent parts of the world [9].

HS is caused by mutations in one of the following genes: SPTA1 (1q21), SPTB

(14q23.3), ANK1 (8p11.21), SLC4A1 (17q21.31) and EPB42 (15q15-q21), that encode the red blood cell (RBC) membrane proteins erythrocytic 1 spectrin alpha chain, erythrocytic 1 spectrin beta chain, ankyrin-1, band 3 anion transport protein, and erythrocyte membrane protein band 4.2, respectively. Defects in these proteins lead to a loss in RBC membrane cohesion and membrane surface area, resulting in erythrocyte sphering, decreased deformability and premature destruction in the spleen sinuses causing it to be hemolyzed immaturely (Figure 2).

Figure 2: Types of mutations in HS



All are inherited as autosomal dominant except alpha spectrin mutation and Protein 4.2 Gene: EPB42, which is usually inherited as autosomal recessive [10].

Advances in conducting diagnostic testing using technologies of next-generation sequencing (NGS) helped a lot in discovering new cases, and new novel mutations as more than 80 ANK1 mutations and 35 SLC4A1 Allelic Variants have been reported in the literature causing HS, and in fact, it is proven to be cost-effective to detect other mutations in different genes linked to HS [11].

The clinical presentations of HS are variable in severity according to the type of mutations ranging from asymptomatic patients discovered during routine lab works or during family screening to very severe presentations of hemolytic anemia requiring blood transfusions. Hemolysis is associated with indirect hyperbilirubinemia,

sometimes with distal tubular acidosis as an association, and gallbladder stone formation and sequelae.

The bone marrow of affected patients will try to compensate for the chronic anemia calling for extramedullary hematopoiesis in spleen and liver with a report of posterior paravertebral mediastinum expansion as a result of the ongoing hematopoiesis [12]. It is also reported as in other hereditary hemolytic anemias, patient with HS are at risk to develop aplastic crises after infection by human parvovirus B19 [13].

HS is also reported with other types of hemoglobinopathies and enzymopathy, causing hemolytic anemia like sickle cell, thalassemia and pyruvate kinase deficiency and the coinheritance of uridine diphosphate glucuronosyltransferase 1A deficiency [14, 15].

Limitations:

We could not send for confirmatory tests for financial reasons as new mutant gene might not be validated unless it is confirmed. Our hospital is not running genetic testing in a local lab and therefore, samples were sent to Bioscientia laboratories.

Conclusion and Recommendations:

A new mutation is reported in this patient causing hemolytic anemia. HS should be in the differential diagnosis of any child with microcytic anemia not responding to iron therapy. We recommend having a national registry for hereditary spherocytosis to discover the most common genetic mutations in Saudi Arabia and describing the course of the disease and its complications in our population.

Consent:

Patient and his family consented before writing the case report.

Conflict of interest:

Author has no conflict of interest and did not receive any funding from any site.

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