Dyskeratosis Congenita with Acute Myeloid Leukemia, Cryptogenic Liver Fibrosis and Portal Hypertension

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Abstract

Dyskeratosis Congenita (DC), a 100-year-old known rare hereditary entity, has recently changed its definition as per the pathogenetic model in the last decade. Now it is well known as one of the telomeropathies, pathognomonically characterized by a triad of reticulate pigmentation of the skin, nail dystrophy, and mucosal leukoplakia. It is a progressive systemic disorder which usually presents with involvement of several family members. Malignancies are increasingly reported. Clinical diagnosis is simple once there is a suspicion, but nowadays genetic diagnosis is advocated. Treatment is symptomatic and organ-oriented. We hereby report an adolescent male who presented with the classical mucocutaneous triad of DC with pancytopenia for four months. Bone marrow examination later revealed evolution of acute myeloid leukemia (AML). Liver function tests, imaging, and liver biopsy showed cryptogenic fibrosis with portal hypertension. Chemotherapy was started since hematopoietic stem cell transplantation was not feasible; however, he died very early due to repeated infections before completion of the treatment. AML and liver disease are increasingly reported independently in DC; however, coexistence of both complications in a single patient at first presentation has never been reported earlier. Early age onset of AML is noticeable too.

Introduction

Dyskeratosis congenita (DC), a multisystem disorder, initially known as inherited bone marrow failure (BMF) syndrome, was first described by Zinsser in 1906, but recognized as a clinical entity by Engman (1926) and Cole (1930). Since then it is also known as ‘Zinsser–Cole–Engman syndrome’. Recently, it emerged as a new entity of ‘telomere biology disorder (TBD)’ or ‘telomeropathy’ which is characterized by a very short telomere (<99th percentile).¹ Till date 11 genes DKC1, TINF2, TERT, TERC, WRAP53 (TCAB1), NOP10, NPH2, RTE1, CTC1, ACD, and PARN have been found to be associated with DC and exhibiting a complex genotype–phenotype relationship.²,³

It is seen mostly in men with a reported annual incidence of less than one per one million populations. Usually it manifests as abnormal muco-cutaneous-nail changes by 10 years of age, BMF by 20 years, and malignancy by 30 years.⁴ After the recognition of its pathogenetic mechanism as telomere dysfunction in the last decade, every system of the body is known to be affected with a greater involvement of high proliferative tissues like skin, bone marrow, immune cells, and intestinal epithelium. The degree of telomere dysfunction is the major determinant of the disease onset and manifestations.⁵ Clinical diagnosis is not difficult once suspicion is high in patients with pancytopenia and pathognomonic muco-cutaneous triad. Nowadays, genetic testing is being considered for confirming the diagnosis with or without use of a screening test i.e. telomere length analysis, which could be false positive. The management requires a multidisciplinary approach with symptomatic organ based treatment.

Hereby, we report this case to share its uncommon and rare associations as well as to review this emerging disorder, especially the pathogenetic model of telomeropathy.

Case Report

A 15-year boy, non-smoker, from the state of Bihar in India, presented with a history of easy fatigability and dyspnea on exertion.
for one month and dry cough with high grade fever including chills and rigors for 15 days. He had no history of jaundice, bleeding from any site, abdominal pain, or chest pain. He had a similar history of fatigue with dyspnea four months prior to this event when he was evaluated outside and found to have pancytopenia with a hypercellular bone marrow. Since then, he had received eight packs of RBC transfusions following which his symptoms improved. He had no history of exposure to toxins. Two other family members were reported to be suffering from similar blood problem and one of whom was transfusion dependent. There were also other diseases present among other family members (Figure 1).

On examination, he was pale and febrile. His weight was 45 kg with BMI of 16.7 kg/m². He had reticulate pigmentation of the skin over the neck along with diffusely distributed depigmented macules over the chest, abdomen, back, palms, and soles. He had oral mucosal leukoplakia along with pigmented macules and dystrophy of nails and toes (Figure 2). Similar skin lesions were also present in many of his family members as shown in the pedigree. This triad of skin, mucosa, and nail changes is classical of DC. The pattern of transmission was autosomal dominant. He had a firm, non-tender spleen palpable 5-cm below the left costal margin. On complete dental evaluation, there was dental caries, losses, and stains.

His hemogram revealed pancytopenia with Hb, 49 g/L; WBC, 1.51 \times 10^9/L with ANC of 0.5 \times 10^9/L; and platelet counts, 80 \times 10^9/L. Peripheral smear revealed a leftward granulocyte shift with few macrocytes. His liver function test (LFT) revealed total bilirubin, 13.68 µmol/L (normal range, 5.0-21.0); albumin, 28 g/L (32-56); globulin, 40 g/L (2.3-3.5); ALT, 0.94 µkat/L (0-50); AST, 0.89 µkat/L (0-50); alkaline phosphatase, 15.68 µkat/L (240-840); and PT, 19s (control PT, 11.8s). These LFT abnormalities were persistent for more than three months suggesting a possibility of chronic liver disease (CLD). His CLD workup for chronic viral hepatitis, Wilson disease, hemochromatosis, and autoimmune hepatitis was negative. Serum B12 and folate were in the normal range. Iron study was suggestive of an iron overload state with transferrin saturation, 98%; ferritin, 2471.70 pmol/L (33-450); and TIBC, 38.66 µmol/L (44.8-80.6). RK-39 for Kala azar was negative. Contrast-Enhanced Computed Tomography of chest and abdomen revealed left side pneumonia and CLD with portal hypertension. This was also supported by an ultrasound doppler study of hepato-portal vein axis. Upper gastro-intestinal endoscopy revealed low grade oesophageal varices. Transient liver elastography (fibroscan) suggested an early portal fibrosis (Score of 8.8 kPa). Bone marrow (BM) aspirate and biopsy showed a hypercellular marrow with granulocyte prominence without any evidence of infection or LD bodies (Figure 3).

Fig. 1: Pedigree of the affected family. Roman numerals indicate generations, squares represent males and females are denoted by circles. Arrow indicates the index case (patient). Diagonal line indicates death

![Fig. 1](image1.png)

![Fig. 2](image2.png)

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(MPO), suggesting acute myeloid leukaemia (AML) (Figure 3). Hence, the patient was diagnosed as a case of Dyskeratosis congenita with early cryptogenic liver fibrosis, secondary hemochromatosis, portal hypertension, AML, and superadded infection as pneumonia.

**Genetic testing**

Detailed pedigree information and peripheral blood samples were collected for molecular investigations after taking an informed consent from the patient and available family members (Figure 1). Karyotype was normal. Genomic DNA was isolated using standard salting out protocol and subjected to PCR amplification of the *TERC, TERT,* and *DKC1* using 100ng DNA, 2.5mM MgCl2, 0.30 mM of each of the dNTPs (Invitrogen, Carlsbad, CA, USA), 20pM of each primer, and 0.5 units of Taq Polymerase (Invitrogen, Carlsbad, CA, USA) in a 25 µL volume mixture using thermocycler ABI 9700 (Applied Biosystems, Foster City, CA). All the amplified products were purified using Qiagen kits (Qiagen, GmbH, Hilden, Germany), sequenced using BigDye Terminator Mix version 3.1 (Applied Biosystems [ABI], Foster City, CA), and analyzed on an ABI-3100 Genetic Analyzer (ABI). Nucleotide sequences were compared with the reference cDNA sequences of *TERC* (GenBank accession number ENSG00000270141), *TERT* (GenBank accession number ENSG00000164362), and *DKC1* (GenBank accession number ENSG00000130826) gene. No pathogenic mutation was identified in these three genes. Hence, other family members were not studied further. However, single nucleotide polymorphisms (SNP) rs2728532 in *DKC1* and rs13167280, rs2075786, rs2853690, rs79662648, and rs2736098 in *TERT* were identified.

During the hospital course, he received two cycles of chemotherapy (Cytarabine, 100 mg/m²/day×24hr infusion×7days and Daunorubicin, 60 mg/ m²/day×3days) for AML. However, there was no significant architecture, mild portal triaditis, focal interface hepatitis, extensive iron deposition in hepatocytes and Kupffers cells, and mild fibrous expansion of few portal tracts which is suggestive of an early hepatic fibrosis with probable secondary hemochromatosis (Figure 4).

Spleen histopathology showed fibro-congestive features. The patient’s haematological profile improved transiently after the splenectomy. However, he became pancytopenic once more after about three weeks. Therefore, he underwent a repeat BM evaluation in view of high suspicion of BMF. Surprisingly it revealed blast cells (20%) with a positivity for CD34 on flow cytometry and positivity for myeloperoxidase (MPO), suggesting acute myeloid leukaemia (AML) (Figure 3). Hence, the patient was diagnosed as a case of Dyskeratosis congenita with early cryptogenic liver fibrosis, secondary hemochromatosis, portal hypertension, AML, and superadded infection as pneumonia.

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**Fig. 3: Photomicrograph of bone marrow biopsy showing hypercellular marrow** (Panel Ax200, H&E). Pancytopenia with occasional blast cells (arrows) are noted in the peripheral blood (Panel Bx400), in hemodiluted bone marrow aspirate (Panel Cx400). Blast cell showing MPO negativity (Panel Dx400) but on Flow cytometry shows MPO positivity (not shown)

**Fig. 4: Photomicrograph of Liver biopsy showing maintained lobular architecture** (Panel Ax40, H&E). There is mild portal triaditis and focal interface hepatitis (arrow) (Panel Bx400, H&E), fibrous expansion of few portal tracts (arrow) (Panel Cx200, Masson trichrome stain), and extensive iron deposition (arrows) in hepatocytes and Kupffers cells (Panel Dx400, Perl’s stain)
response and the hospital course became stormy with superadded neutropenic enterocolitis and hospital-acquired pneumonia, which were treated. In the meantime, he developed another episode of pneumonia; despite aggressive and appropriate antibiotic therapy, he succumbed to his illness.

**Discussion**

Telomeropathy, a disease of telomere maintenance machinery, is an emerging spectrum disorder well known to the world after discovery of the telomere by Nobel prize winners Blackburn, Greider, and Szostak (2009). DC is known to exist since the last century; however, recent new look into its pathogenesis involving the telomere dysfunction has attracted medical professionals. Clinical diagnosis of DC is considered when at least two of four major features (mucocutaneous triad and BMF) and at least two multisystem features are present (Table 1).6 Our case had classic triad with liver disease, teeth abnormality, and malignancy as systemic features.

For a case definition, individuals with characteristic clinical findings having very short telomeres and/or a mutation in one of the DC-associated genes should be considered. Telomere length testing is usually done by flow-FISH which is considered the best having sensitivity of 97%, specificity of 91%, and positive predictive value of 85%. Yet, there is false positivity as seen in Schwachman–Diamond syndrome and Fanconi anaemia. Therefore, genetic analysis should be performed in such cases. Cancer is more common in DC caused by TERT and TERC, intermediate by DKC1, and least common by TINF2 gene mutation.9 Therefore, in the present case study genetic screening for TERT, TERC, and DKC1 was undertaken but no pathogenic lesions were identified in these genes. Only six SNPs were identified in TERT and DKC1. TINF2 molecular screening is ongoing. Pediatric patients of sporadic AML have not shown any positivity to TERT/TERC mutations in contrast to adults.10 Hitherto, gene mutations are more of a risk factor than etiologic factors for the disease and till date, mutations have been identified in about 60-70% of DC cases. Variable expression is seen in families wherein some members have typical skin lesions and some have isolated BMF syndromes or malignancies without skin features; while others do not present with similar features exhibiting variable penetrance. Children often have multisystem involvement, but adults show much variability and may present with a single major feature. In pre-molecular diagnostic era, patients were classified based on inheritance patterns into X-linked, autosomal dominant, or autosomal recessive; however, availability of clinical genetic testing has shown that the inheritance of DC may be more complex. Since telomere length is heritable and exhibits anticipation, subsequent generations show increased severity and an early age of onset of manifestation as seen in our pedigree.5 Prenatal testing for pregnancies at increased risk is possible if a specific mutation is known.11

Our case had pancytopenia, splenomegaly, and mucocutaneous involvement, therefore we thought of two inherited BMF syndromes. One is DC which has greater emphasis in the ectodermal picture while skeletal and renal anomalies are more prominent in Fanconi’s anaemia; besides, splenomegaly is not a feature of later one.12 Bone marrow picture in DC ranges from hypercellular to normocellular to hypocellular. In our case within very short time span i.e. one month, BM picture changed from hypercellular to leukemic changes without going to end stage BMF which is one of the uniqueness of this case. Allogenic-HSCT is the only curative treatment available for BMF or leukemia, but there is associated high toxicity including risk of malignancies, even after reduced-intensity conditioning regimens.4

**Malignancies are being increasingly reported in about 10 to 15% cases of DC. Telomeropathy was initially thought to be protective for cancer development due to decreased telomerase activity, later on proved to be having increased risk due to the genotoxic stress by persistent DNA damage signals at the telomeres, telomere fusions, and genomic rearrangements driven by telomere repair-mediated recombination.13 The DC Registry of United Kingdom (1995 onward) and the National Cancer Initiative’s DC cohort (2002 onward) are two large disease databases providing information on most of the cancers. These data and other case reports have showed head and neck cancer being most common type with only few registered cases of AML but with an approximately 200-fold odd ratio.5 Their average age of presentation of AML was 30 years but our case was only 15 years of age. This is possibly due to the genetic anticipation of an autosomal dominant transmission.

<table>
<thead>
<tr>
<th>Major clinical features</th>
<th>Other recognized somatic features</th>
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<tbody>
<tr>
<td>Mucocutaneous triad</td>
<td>Epiphora</td>
</tr>
<tr>
<td>Abnormal skin pigmentation</td>
<td>Learning difficulties/developmental delay/</td>
</tr>
<tr>
<td>Nail dystrophy</td>
<td>Mental retardation</td>
</tr>
<tr>
<td>Leukoplakia</td>
<td>Pulmonary disease</td>
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<tr>
<td>Bone marrow failure</td>
<td>Short stature</td>
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<tr>
<td>Deafness</td>
<td>Extensive dental caries/loss</td>
</tr>
<tr>
<td>Osteoporosis/aseptic necrosis/scoliosis</td>
<td>Esophageal stricture</td>
</tr>
<tr>
<td>Urethral stricture/phimosis</td>
<td>Premature hair loss/graying/sparse eyelashes</td>
</tr>
<tr>
<td>Microcephaly</td>
<td>Hyperhidrosis</td>
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<tr>
<td>Short stature</td>
<td>Malignancy</td>
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<tr>
<td>Intrauterine growth retardation</td>
<td>Liver disease/peptic ulceration/enteropathy</td>
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<tr>
<td>Ataxia/cerebellar hypoplasia</td>
<td>Hypogonadism/undescended testes</td>
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**Table 1: Multisystem clinical features of Dyskeratosis congenital**
as shown in the pedigree. To the best of our knowledge, this is the first case report from India with DC having three malignancies in one family. AML is not reported in DC from India, but single case of acute lymphoblastic leukemia (ALL) has been reported\textsuperscript{14}. In our case, we had started chemotherapy and HSCT was not possible due to lack of the non-carrier sibling. Post-transplant (with low intensity Fludarabine based protocol) survival has been documented to be 10 to 72 months in BMF cases; however, no survival has been reported in AML cases.\textsuperscript{11,13}

Liver diseases in DC are not common but detection rate has increased among the family members. Its spectrum may range from asymptomatic LFT abnormality as in our case to cirrhosis of the liver. Early screening may help in detection of more cases. Histologically, there is also a spectrum of manifestations in form of steatosis, hepatitis, early fibrosis to cirrhosis, and hemosiderosis (more often due to frequent use of iron tablets and blood transfusions).\textsuperscript{15} Our single case is having a similar picture with steatosis, inflammation, necrosis, fibrosis, and iron accumulation though not cirrhosis. From India, one case of DC with CLD (image proven) & portal hypertension without any malignancy has been reported.\textsuperscript{16}

There are usually repeated infections due to the telomere dysfunction causing an aberrant immune system. Our case was having recurrent pneumonia and enterocolitis. Infective pneumonia should be differentiated from idiopathic pulmonary fibrosis (IPF), which is the most common and serious lung involvement in DC and usually seen after HSCT.\textsuperscript{2} Lung transplant is the only available treatment in IPF.

Until now, the recommended treatment is only supportive and organ-oriented with the care of all family members including genetic counseling and avoiding toxic agents like smoking, alcohol, or non-leucodepleted or non-irradiated blood products. Future possibilities of telomerase therapeutics (gene therapy) and vaccines are there in genetic variant syndromes. There is a society group with a website named, www.dcoutreach.org, which maintains accounts on Twitter, Pinterest, and Facebook for DC outreach group facilitating an interaction among different family members and professionals.\textsuperscript{3}

**Conclusion**

In summary, we describe a case of pathognomonic mucocutaneous triad of DC who presented with AML and CLD, refractory to the existing medical treatment. This is the first report of coexistence of both complications in DC. Simultaneously, we learn the genetic approach to a telomeropathy, although the mutations were not detected in any of the tested genes. This case also give us the opportunity to learn more about the telomeropathy as a family diagnosis and DC as an individual diagnosis.

**Authors’ contributions**

PKP had given the concept, searched literatures, analyzed and drafted the work, RS had analyzed and critically revised the work, KK had collected data and drafted the work, and RJ had interpreted and drafted the work. AS and SB had done genetic analysis, given their data, and drafted the work. PM had interpreted, especially hematological data and critically revised the work. TK had collected pathological data and drafted the work. PKP, RS, RJ, KK, and PM were the physicians involved in the patient management. All authors read and approved the final manuscript.

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**References**