

A Case of Kyphoscoliotic Ehlers-Danlos Syndrome

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Abstract

During a post-mortem examination multiple pathological findings were discovered in an individual known to have suffered from Ehlers-Danlos Syndrome (EDS). The most prominent of these findings was severe curvature of the spine in both coronal and sagittal planes, suggestive of the rare Kyphoscoliotic Subtype of EDS (kEDS). Cardiac abnormalities included a rare quadricuspid pulmonary valve. Lung and bone irregularities, bilateral diaphragmatic hernias, and long segment bowel stricture were also identified. Immunohistochemical staining of skin, muscle, and lung tissue revealed atypical composition and the reduced presence of lysyl hydroxylase (PLOD1). Here we describe autopsy findings of a kEDS case and we discuss the implications of kyphoscoliosis on the heart and the lungs.

Introduction

Ehlers-Danlos Syndrome (EDS) is a group of connective tissue disorders characterized by the common features of joint hypermobility, hyperextensible skin, and tissue fragility [1,2]. As updated in the 2017 International Classification for the Ehlers-Danlos Syndromes, there are now 13 subtypes of EDS, based on major and minor clinical criteria and molecular testing [1,2]. One of these subtypes of EDS is the kyphoscoliotic form, hereafter referred to as kEDS [3]. Major clinical criteria of kEDS include generalized joint laxity, severe muscle hypotonic at birth, progressive scoliosis typically present at birth, scleral fragility and rupture of the ocular globe [1,2]. Minor clinical criteria of kEDS include tissue fragility with atrophic scars, easy bruising, arterial rupture, marfanoid habitus, microcornea, radiological considerable osteopenia, and a positive family history [1,2]. Intelligence is generally unaffected and life span can be normal. Cardiopulmonary complications may include rupture of medium-sized and restrictive respiratory disorders due to kyphoscoliosis if severe may shorten life span [4].

Two distinct biallelic mutations have been identified in kEDS patients. In the majority of kEDS cases, mutations are present in *PLOD1*, the gene encoding the lysyl hydroxylase enzyme (also known as procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1 or *PLOD1*), leading to abnormal collagen formation [4] and processing [5]. In these cases, laboratory diagnosis of kEDS can be made by a demonstration of a markedly increased ratio of deoxypyridinoline to pyridinoline crosslinks in the urine measured by high-performance liquid chromatography (HPLC) and/or by measurement of lysyl hydroxylase levels in skin fibroblasts [6]. Genetic testing for mutations in *PLOD1* can also be used for the differential diagnosis of kEDS [2,6]. In other kEDS patients, mutations are present in *FKBP14*, a gene encoding an isomerase protein that is believed to accelerate protein folding in the endoplasmic reticulum [7] and is known to interact with select collagens [8]. Although the prevalence of kEDS is unknown, this rare autosomal recessive disorder is estimated to affect 1 in 100,000 live births [4]. Here we report the multiple pathological findings upon autopsy of an individual who had kEDS.

Case Report

The body of a 62 year old deceased male reported to have suffered from EDS was donated to the University of South Dakota Sanford School of Medicine (USD-SSOM) Body Donation Program. Donor consent was obtained to dissect the cadaver for educational and research purposes. Conventional dissection [9] was performed at USD-SSOM.

Autopsy of the EDS case revealed the presence of multiple anatomical pathologies. The most striking external visible feature was severe curvature of the spine in both coronal and sagittal planes (i.e., kyphoscoliosis; Figure 1A). Upon inspection of the thoracic and abdominal cavities, the profound kyphoscoliosis was accompanied by erector spinae and transverse spinal muscle deformity. The presence of kyphoscoliosis in a case known to have had EDS supports our diagnosis of kEDS.

Examination of the femoral head and neck, acetabulum, radial notch of the ulna, and medial epicondyle showed evidence of reduction in bone density based on visual inspection of the bony and articular surfaces. Fat adhesions were present on the left anterior cruciate ligament.

Diaphragmatic hernias measuring 3 cm on the right and 2 cm on the left were present bilaterally along the posterior mediastinum. The right hernia sac contained large intestine and part of the greater omentum. The left hernia sac contained greater omentum and part of the stomach. The thoracic cavity was restricted with decreased lung size bilaterally (Figure 1B). Inspection of the small and large intestine identified a 5 cm long segment stricture of the right colon, presumed to be the ascending colon.

The chest wall contained median sternotomy wires and displayed features of pectus carinatum. Upon inspection of the heart, it was noted that the heart was globular. There was gross cardiomegaly relative to the body size. The right atrium was normal in size, but with post-operative changes and hypertrophied. An implanted tricuspid annuloplasty ring was identified in the appropriate position. Mild right ventricular hypertrophy was also present. There was an area in the mid-anterior right ventricular outflow tract where the right ventricular anterior wall lost its muscular appearance. The outflow tract thinned significantly to about 1 mm and was translucent with a fibrous appearance. The translucent area was approximately 3x4 cm.

The pulmonary valve appeared native; however, there were four distinct cusps with matching sinuses (Figure 1C). The pulmonary valve leaflets were translucent and not dysplastic with good leaflet apposition. The main pulmonary artery wall was thinned, especially anterior.

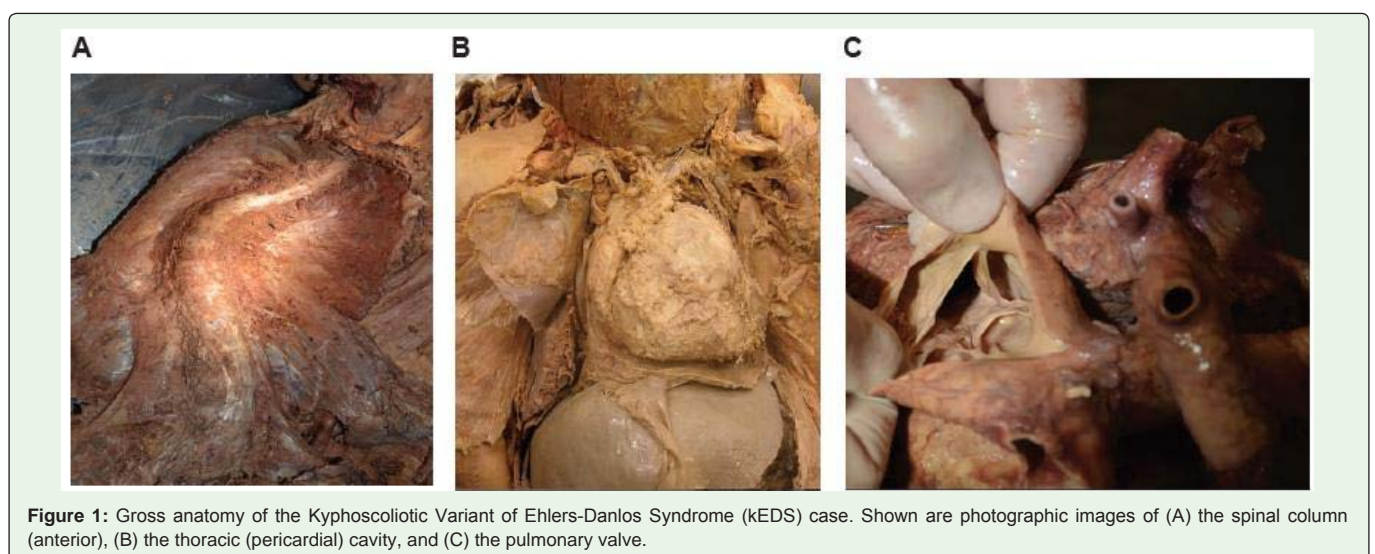
The left atrium was dilated. The left atrial appendage appeared longer than normal and somewhat hypertrophied. There was a prosthetic bi-leaflet valve in the position of the mitral valve. The left ventricle appeared to be abnormally hypertrophied. Former papillary insertions could not be identified. The left ventricular cavity size appeared small for the body size.

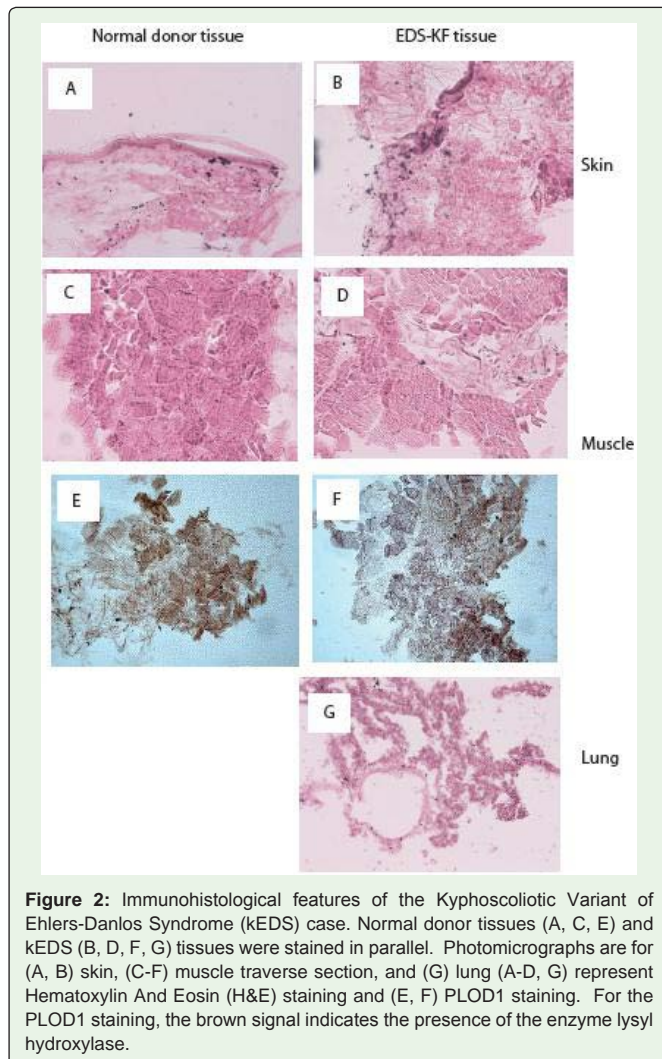
There was a prosthetic bi-leaflet valve in the aortic valve position.

Permanent pacemaker wires entered the left subclavian vein, one implanted in the mid-anterior right atrium, and the other implanted through the tricuspid valve into right ventricular trabeculations. Both leads were firmly fixed with endothelialization.

To elucidate the histopathology, we harvested lung tissue, skin from the medial aspect of the forearm, and muscle tissue from the brachioradialis of the kEDS case and these same tissues from an age-matched control male cadaver. Tissue sections were fixed in 1% formalin, dehydrated using serial ethanol washes, embedded in paraffin, cut in 15µm sections using a microtome, and slices placed on Superfrost Plus glass slides. Subsequently, tissues were treated with citric acid antigen retrieval buffer in a steamer, blocked with blocking buffer, permeabilized, and then incubated with anti-*PLOD1* antibody (200 µg/ml, Sigma-Aldrich) at 4°C for 48 hours. Next, the slides were washed, incubated in secondary anti-rabbit antibody for 1 hour, washed, and transferred to a DAB/H₂O₂ solution for 10 minutes. Slides were then cover slipped. On separate slices, Hematoxylin and Eosin (H&E) staining was performed and the prepared slides were examined and imaged using a light microscope. Histological features, particularly the tissue architecture and organization of collagen fibers, were assessed by three different observers in a blinded manner.

In comparison to tissue specimens from the control subject, the kEDS tissue specimens exhibited distinct features. As revealed by H&E staining, the kEDS skin tissue featured blood extravasation in atrophic dermis, abundant hemosiderin, and a whorled pattern of collagen specifically around the blood vessels (Figure 2A and 2B). Marked atrophic changes were apparent in the kEDS muscle tissue (Figure 2C and 2D). These atrophic features were associated with reduced levels of lysyl hydroxylase in the kEDS muscle tissue as determined by immunohistochemical staining with anti-*PLOD1* antibody (Figure 2E and 2F). The reduced *PLOD1* levels were variable throughout the muscle tissue, with some cells completely lacking the enzyme and other cells having relatively normal levels. H&E staining of the kEDS lung tissue revealed the presence of pulmonary hemorrhage and heart-failure cells (hemosiderin-laden macrophages) (Figure 2G).





We also attempted to identify the underlying genetic mutation in (*PLOD1*). The *PLOD1* gene encompasses 41.3kb of chromosome 1 and contains 19 exons. Introns 9 and 16 are rich in Alu sequences and are therefore prone to intragenic homologous recombination. Nonetheless, at least 20 different mutations in *PLOD1* have been associated with kEDS [10]. We successfully extracted genomic DNA from the kEDS tissue using a commercial kit designed for the isolation of DNA from formalin-fixed tissue (Zymo Research). Most of the recovered DNA was approximately 300bp in length as determined by agarose gel electrophoresis. Because of the high degree of fragmentation, we were discouraged from analyzing the entire gene by sequencing overlapping PCR products. Instead, we focused on the large duplication that is one of the more prevalent mutations among kEDS cases [10]. Standard PCR was performed using a pair of oligonucleotide primers (JH71: 5'-CAGTTTTACAACATTCATATC-3' and JH55:5'-GATCACGAGGTCAGGAGAT-3') as previously described [11] to screen for the large duplication mutation in the *PLOD1* gene. The JH71 and JH55 primers are specific for the 3' end of exon 16 and the 5' end of exon 9, respectively. In the presence of the seven exon duplication in *PLOD1*, these primers should amplify a 300 bp fragment. Using the JH71/JH55 primer pair, we were unable to

amplify a 300 bp fragment with DNA from the kEDS case (data not shown). This result can suggest that the kEDS case had a different mutation in *PLOD1* or perhaps a mutation in *FKBP14*.

Discussion

In this case study, we identified the presence of skeletal, thoracic, and cardiac abnormalities suggestive of the severe clinical sequelae of kEDS (Figure 1). In addition, muscle tissue from the case was determined to have reduced levels of the enzyme lysyl hydroxylase (Figure 2) which is specific for kEDS.

The internal pathological findings in our case were dominated by cardiac manifestations: cardiomegaly, tricuspid valve pathology requiring tricuspid annuloplasty with ring placement, aortic and mitral valve pathology requiring prosthetic valve placement, quadricuspid pulmonary valve, thinned right ventricular outflow tract and pulmonary arterial wall (both anterior and not appearing to be post-surgical changes), right and left ventricular hypertrophy, dilated left atrium, and pacemaker placement. Whether the atrioventricular pacemaker was placed for native rhythm issues or for post-operative sequelae cannot be determined in this case.

Mitral Valve Prolapse (MVP) with or without regurgitation has been recognized in EDS patients [12]. The suspected cause of mitral insufficiency is due to poor tissue integrity and the lack of sufficient support of the mitral valve leaflets [6]. Aortic regurgitation can be subsequently found for reasons similar to mitral insufficiency. We postulate that mitral and aortic valve pathology was severe enough to warrant replacing both valves. Although MVP is well recognized with EDS, it rarely requires surgical replacement of the mitral valve [13]. Nonetheless, a unique surgical replacement of the mitral valve has been described for a 40 year-old patient with kEDS suffering from severe mitral and mild aortic regurgitation [13].

Our finding a native quadricuspid pulmonary valve is rare. The prevalence of a quadricuspid pulmonary valve in postmortem examinations ranges from 1 in 1,000 to 1 in 4,000 [14,15]. The quadricuspid pulmonary valve in our patient was composed of 4 cusps of equal size. There have been case reports of quadricuspid valves with evidence of severe pulmonary regurgitation and stenosis [16]. However, the morphology of the quadricuspid pulmonary valve encountered in our case along with the fact that no intervention was undertaken to fix the valve suggests that this quadricuspid pulmonary valve was a clinically insignificant finding. To our knowledge this is the first case in the literature reporting a quadricuspid pulmonary valve in an individual with EDS of any subtype. A quadricuspid pulmonary valve and left pulmonary artery aneurysm were reported in an asymptomatic 58-year-old patient after an uncomplicated ablation procedure of an AV nodal re-entry tachycardia [17]. That patient's chest radiograph did not show scoliosis [17].

The combination of a quadricuspid pulmonary and a bicuspid aortic valve has been described. These may have been caused early in development by abnormal proliferations in the common trunk and abnormal fusion of the aortopulmonary septum [18]. We cannot determine the native aortic valve anatomy in this case.

Collagen abnormalities might be responsible for thinning of the anterior right ventricular outflow tract and the anterior main pulmonary artery. It is difficult to ascertain whether these cardiac manifestations were a result of intrinsic collagen abnormalities or

from the kyphoscoliosis, since the case was examined post mortem with a very little clinical history available. Heart defects such as MVP are common in non-kEDS patients indicating that a variety of collagen abnormalities can contribute to cardiac abnormalities. Our case demonstrates the spectrum of cardiac manifestations that can occur in patients with kEDS.

Additionally, on gross examination the lungs looked significantly consolidated and the lung volumes seemed reduced. The combination of the severe kyphoscoliosis and the cardiomegaly likely resulted in considerable restrictive type pattern of pulmonary function. The kyphoscoliosis in this case appears to have caused a substantial amount of stress within the chest cavity, contributing to the many pathological changes described in the heart and the lungs and leading to heart failure. This observation is backed our findings of hemosiderin-laden macrophages or heart-failure cells that were observed in the lung tissue upon microscopic analysis (Figure 2).

Additional observations included a long segment bowel stricture (thought to be the sigmoid colon), bilateral diaphragmatic hernias, and reduction of bony density in the femoral head and neck, acetabulum, radial notch, and medial epicondyle. It is unclear whether the bony abnormalities or osteopenia and ACL pathologies are specifically attributable to the kEDS or general aging.

A limitation of this study was that we were unable to obtain a complete medical history for this autopsy case to appreciate the longitudinal nature of his disease and when surgeries occurred. In addition, the findings indicated severe cardiopulmonary disease, although the exact cause of his death is unknown.

In summary, our findings reveal the wide variety of cardiac and lung sequelae that can accompany the kyphoscoliotic form of Ehlers-Danlos syndrome. Moreover, the surgical treatments in this case can raise awareness for the clinical management of kEDS patients.

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