Case Reports in Dermatology

Case Rep Dermatol 2022;14:283–293
DOI: 10.1159/000525068
Received: January 17, 2022
Accepted: April 17, 2022
Published online: October 6, 2022

Single Case

Epidermolysis Bullosa and Rickets in a 21-Year-Old Female: A Case Report

Renni Yuniati[a] Rakhma Yanti Hellmi[b] Gema Citra Dwijayanti[c] Meira Dewi Kusuma Astuti[d] Gerard Pals[e] Dimitra Micha[e] Sultana MH Faradz[f]

[a]Department of Dermatovenerology, Faculty of Medicine, Diponegoro University, Semarang, Indonesia; [b]Division Rheumatology, Department of Medicine, Dr. Kariadi Hospital, Semarang, Indonesia; [c]Department of Medical Biology, Jenderal Soedirman University, Purwokerto, Indonesia; [d]Department Pathology, Dr. Kariadi Hospital, Semarang, Indonesia; [e]Department of Human Genetics, Amsterdam Movement Sciences, Vrije Universiteit Amsterdam, Amsterdam UMC, Location VU University Medical Center, Amsterdam, The Netherlands; [f]Division Human Genetics, Center for Biomedical Research, Faculty of Medicine, Diponegoro University, Semarang, Indonesia

Keywords
Epidermolysis bullosa · Rickets · Vitamin D deficiency · Bone bowing · Indonesia

Abstract
Epidermolysis bullosa (EB) is a group of rare genetic diseases that exhibit mechanical fragility of the skin. This condition will result in the occurrence of skin blisters, skin erosions, and skin ulcerations when the skin is subjected to trauma. In this case report, we present a case of EB and multiple skeletal deformities in a 21-year-old female. She came to our clinic with recurrent skin exfoliations and blisters that occurred since she was 4 years old and multiple bones bowing since she was 9 years old. On physical examinations, we found generalized hypopigmentation macule with erythematous skin. There were numerous bullae and crusted lesions, with erosion and excoriations on the lesions. Laboratory examinations identified low vitamin D 25-OH (8.6 ng/mL). Bone densitometry measurement found low bone density, and X-ray examination found osteopenia and bone bowing. Using whole-exome sequencing, no causative pathogenic sequence or copy number variants in the genes associated with Mendelian inherited disorders were detected. The low levels of vitamin D 25-OH may most likely be the main reason for the occurrence of rickets in this patient aside from the genetic disorder.

Correspondence to:
Sultana MH Faradz, sultanafaradz@gmail.com

Case Rep Dermatol 2022;14:283–293
DOI: 10.1159/000525068

Introduction

The mechanical fragility condition in epidermolysis bullosa (EB) will result in the occurrence of skin blisters, skin erosions, and skin ulcerations when the skin is subjected to even the slightest mechanical trauma [1, 2]. In severe forms of EB, this mechanical fragility of the skin extends to any epithelial-lined organ and may cause skin malignancy, therefore significantly reducing patients' life expectancy. Patients with EB are more likely to have low levels of hemoglobin, iron, vitamin D, zinc, and albumin [3].

Rickets often occurs along with osteomalacia, which refers to the impaired mineralization of the bone matrix. One of the most common and essential factors underlying the occurrence of rickets is impaired vitamin D levels [4]. Vitamin D is important for the absorption of calcium from the intestinal tract. This will cause hypocalcemia and/or hypophosphatemia, which disturbs bone formation due to impaired mineralization [5]. Treatment of hypovitaminosis D can also reduce the inflammation and blistering that occurrs in EB by suppressing immune complex-induced reactive oxygen species production [6].

Case Report/Case Presentation

In this case report, we present a case of EB and multiple deformities mainly on the extremities of a 21-year-old female. The patient came to our Dermatology and Venereology Clinic, Kariadi General Hospital of Central Java province, a tertiary-level hospital. She had a main complaint of recurrent skin blister episodes and broken bones. She had her first skin blistering episode when she was 4 years old. All of the patient's skin was blistered and exfoliated, and there was a minimal amount of intact skin present at that time. She was brought to primary health care in the village of Central Java province and was given ceramide ointment treatment. After the ointment was applied, the wounds healed and dried. She is the first daughter and the third child in her family (shown in Fig. 1).

When she was 9 years old, she had blisters developed almost on her entire body surface, which peeled off with foul-smelling discharge along with joint pain. She was given oral

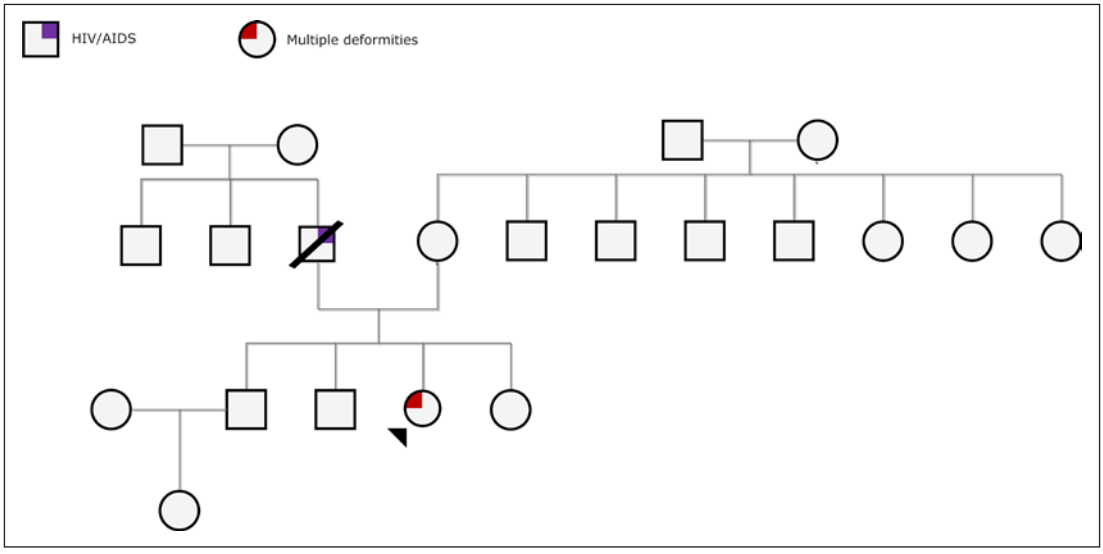


Fig. 1. Pedigree of the index patient in our case.

Case Rep Dermatol 2022;14:283–293
DOI: 10.1159/000525068

medication for her skin blisters and joint pain by the primary health care in the village. When she was around 10 years old, the same symptoms erupted again, which caused her not to be able to wear any clothes because the clothes stuck to the skin. She was given the same therapy as the one given for her skin blister; however, the therapy only resolved her dermatological problems. Within the next few years, she often experienced fever and edema in both lower extremities.

When she reached 9 years old age, her condition gradually worsened so she had to start using cane to walk. Soon after, she had fallen when she was walking; then she had bowed on the leg, which prevented her from going to school. In 2018, she was hospitalized at the Kariadi Hospital Semarang due to lower extremity bone bowing with presumptive diagnosis of Paget's disease and differential diagnosis of EB. She was again rehospitalized in 2021 due to swelling and joint pain, followed by bone weakness, which caused her not to be able to walk.

She only experienced her menstrual period once in 2019. The menstrual period lasted for only 3–4 days, with minimal amount of menstrual blood (approximately 66 mL). There were no significant events in her prenatal and perinatal history. Her birth weight was 3,100 g. There are no other family members with a similar condition and no history of consanguineous marriage. Her father passed away because of HIV/AIDS.

On physical examinations, we found partial alopecia and hair squama, stomatitis, and dental caries with nonexistent axillary and pubic hair and minimal breast development (Tanner stage 3). On skin examinations, we found generalized hypopigmentation macule with erythematous skin. There were numerous bullae and crusted lesions, with erosion and excoriations on the lesions. We also found hyperpigmented and hypopigmented lesions (shown in Fig. 2) on almost all skin regions (scalp, face, chest, hands, feet, and back area). We also found onycholysis on all of her nails. Examination of the extremities revealed that she has diminished muscle strength on the superior extremities and no muscle strength on the lower extremities. All of her muscles had a weak tone. Clinically, arm and leg bowing were found in this patient, most likely due to vitamin D3 deficiency. In 2018, when she was 19 years old, we examined several radiological tests for this patient. The plain chest posteroanterior radiograph showed that she had osteoporotic bones and cardiomegaly. The plain vertebrae radiograph

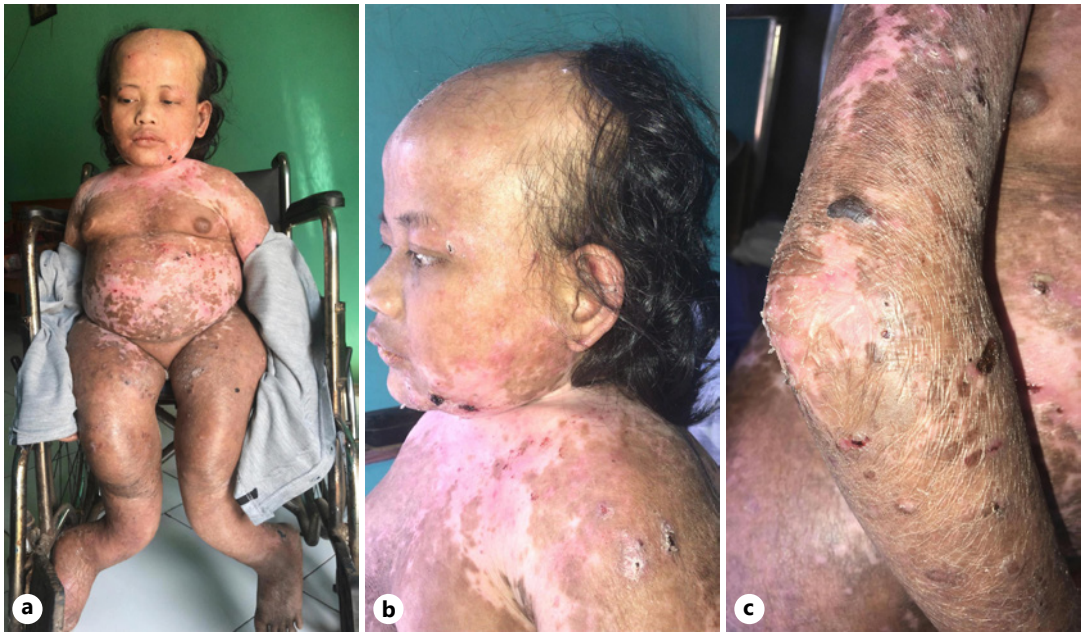


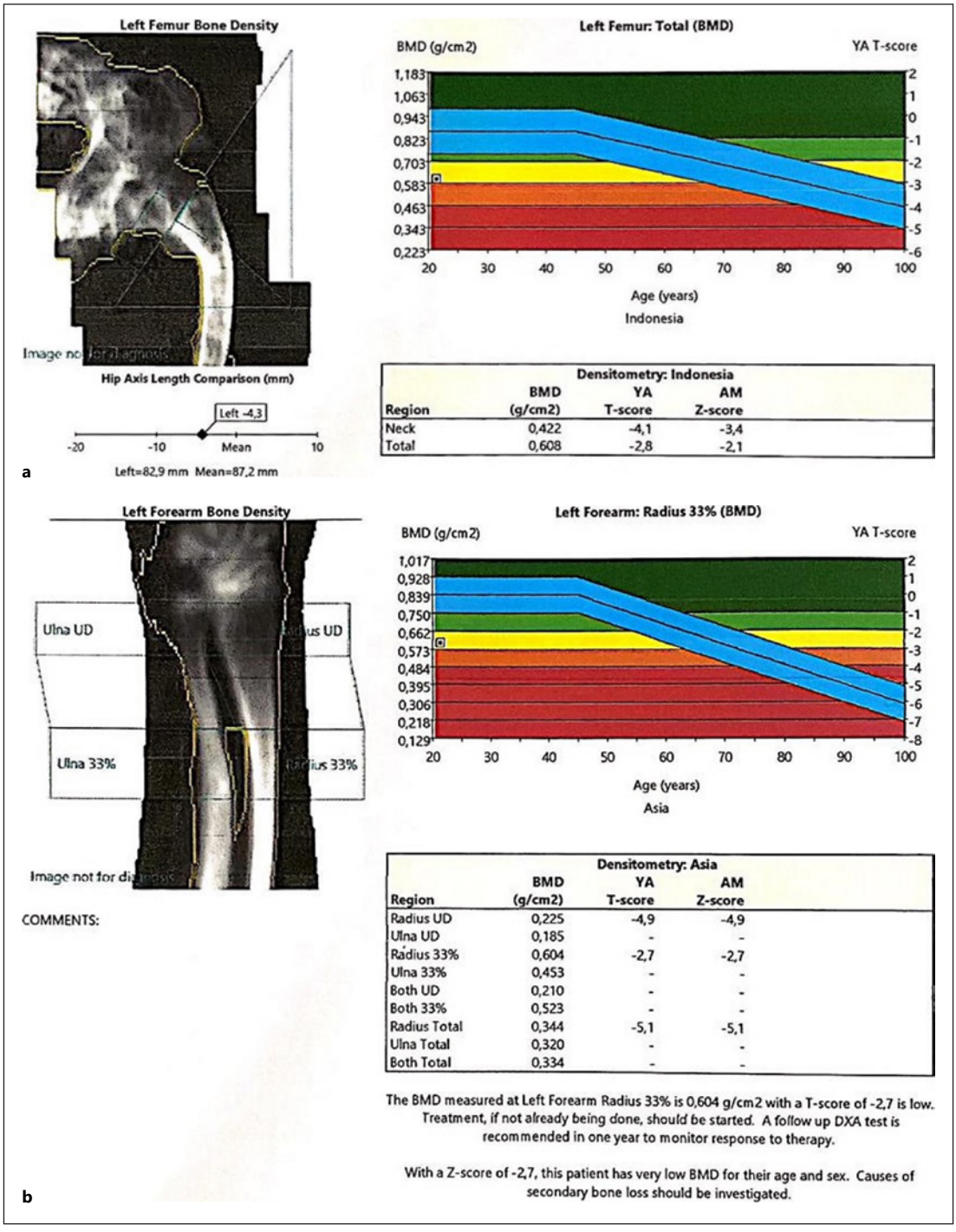
Fig. 2. a–c Clinical picture of our patient. Note the blistering of the skin and bowed leg bones.

Case Rep Dermatol 2022;14:283–293
DOI: 10.1159/000525068

shows osteoporotic bone systems, vertebral scoliosis, and flattened/compressed vertebral bodies, albeit with no intervertebral disc compression. The plain inferior extremity radiograph showed that she had osteopenia with arthritis of the knee joints.

Bone Mineral Density Examination

When the patient was 21 years old, we conducted a bone mineral density (BMD) examination and skeletal survey examination (shown in Fig. 3). On BMD examination, we found



3

(Figure continued on next page.)

Case Rep Dermatol 2022;14:283–293
DOI: 10.1159/000525068

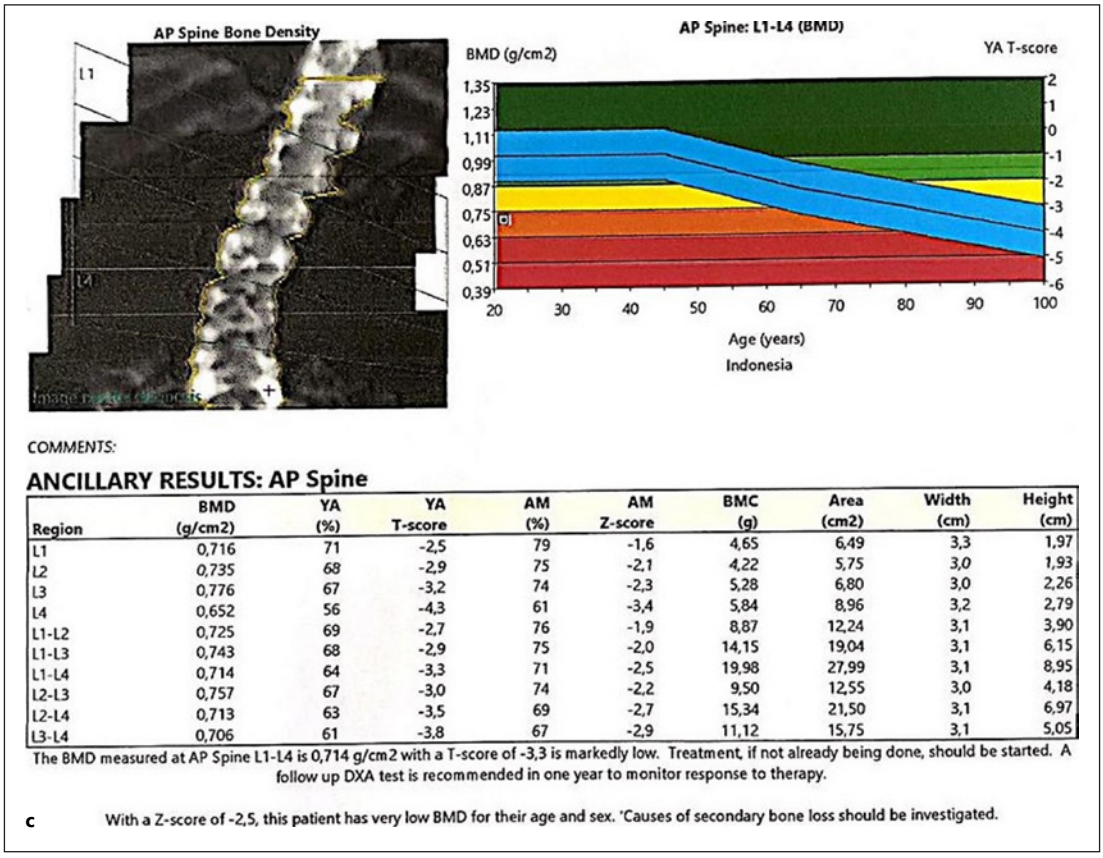


Fig. 3. Bone mineral densitometry results. **a** Left femur bone density. The BMD measured at the left femur neck is 0.422 g/cm^2 with a *T*-score of −4.1, which is severely low. With a *Z*-score of −3.4, this patient has a very low BMD for her age and sex. The BMD measured at the left femur total is 0.608 g/cm^2 with a *T*-score of −2.8, which is low, severely low. Treatment, if not already being done, should be started. With a *Z*-score of −2.1, this patient has a very low BMD for her age and sex. The causes of secondary bone loss should be investigated. **b** Left forearm bone density. The BMD measured at the left forearm radius 33% is 0.604 g/cm^2 with a *T*-score of −2.7, which is low. With a *Z*-score of −2.7, this patient has a very low BMD for her age and sex. **c** AP spine bone density. The BMD measured at the AP-spine L1–L4 is 0.714 g/cm^2 with a *T*-score of −3.3, which is markedly low. Treatment, if not already being done, should be started. With a *Z*-score of −2.5, this patient has a very low BMD for her age and sex. The causes of secondary bone loss should be investigated.

scoliosis on the thoracic and lumbar vertebrae with left convexity, sclerosis of the head and neck of the left femoral bone, sclerosis of the one-third of the medial surface of the left radial bone, and sclerosis of the left ulnar bone. BMD tests showed that she has radius UD BMD of 0.225 g/cm^2, with a *T*-score of −4.9 and *Z*-score of −4.9. The BMD examination showed that she has BMD below the expected range for her age.

Radiologic Findings

On cranium X-ray examination, we found that she has osteopenic bone structure, nasal bone depression, and diploic space thickening on the left parietal region, with multiple linear opacities that show bone remodeling. The cephalic index calculation was 86.7 (brachycephalic; female reference range: 71.0–90.4), and the modulus was at 13.15 (microcephaly; female reference range: 15.7–18.5).

On thorax and thoracolumbar spine X-ray examination, we found that she has osteopenic bone structure and scoliosis on the thoracic and lumbar vertebrae with left convexity. On

Case Rep Dermatol 2022;14:283–293
DOI: 10.1159/000525068

Table 1. Radiographic examination

Test	Age when tested	Results
Plain chest posteroanterior radiograph	19 years	Osteoporotic bones Cardiomegaly
Plain vertebrae radiograph	19 years	Osteoporotic bone systems Vertebral bodies are flattened/compressed No intervertebral disc compression found Scoliosis of vertebrae
Plain inferior extremity radiograph	19 years	Osteopenia with arthritis of the knee joints

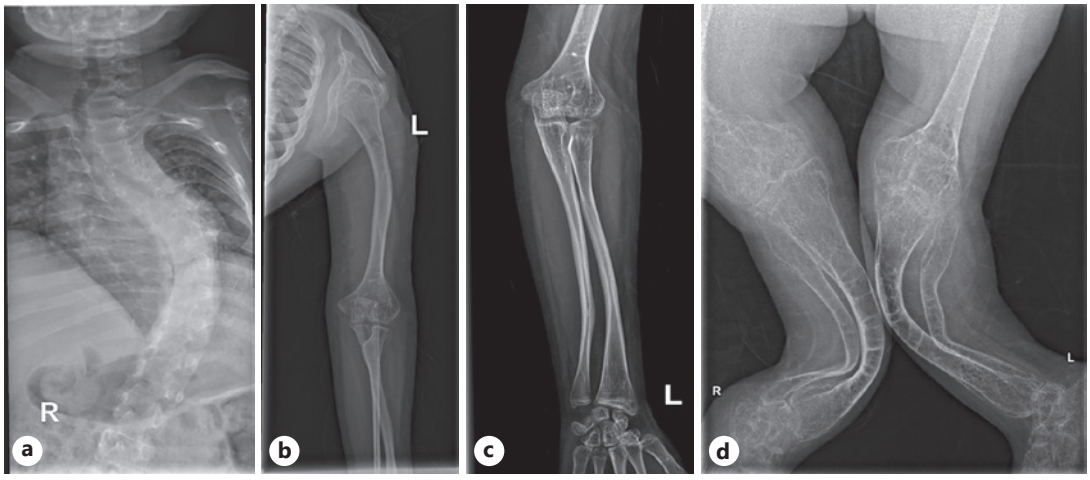


Fig. 4. X-ray findings of our patient. **a** Scoliosis on the vertebrae. **b**, **c** Bowing of the arm bones. **d** Bowing of the leg bones.

pelvic X-ray examination, we found osteopenic bone structure and visualized bowing of bilateral proximal femoral bones. On upper extremity X-ray examination, we found osteopenic bone structure and visualized bowing of the humerus, radius, ulna, and clavicles. We also found a small and sharpening of the right distal phalanges of fingers 1–5 and the left distal phalanges of fingers 2–5.

On lower extremity X-ray examination, we identified osteopenic bone structure with cortical thinning; visualized bowing of both femoral bones, forming saber shin deformity appearance with multiple linear opacities on the one-third proximal shaft of the femur, tibia, and fibula; visualized bowing with multiple linear opacities with a bamboo crane appearance on both shafts of tibial bones; visualized deformities with cortical thinning on both distal epi-metaphyseal femoral bones and both proximal epi-metaphyseal proximal tibia and fibula, and narrowing of the right knee articular space and left superposition; and visualized narrowing of both proximal-distal interphalangeal joints of toes 2–5. The X-ray findings are shown in Table 1 and Figure 4.

Anatomical Pathology Analysis

We conducted a skin biopsy and anatomical pathology examination with a sample taken from the left upper arm (shown in Fig. 5). We found that the tissue consisted of squamous stratified keratinized epithelium, with hyperkeratosis and hypergranulosis. Some of the

Case Rep Dermatol 2022;14:283–293
DOI: 10.1159/000525068

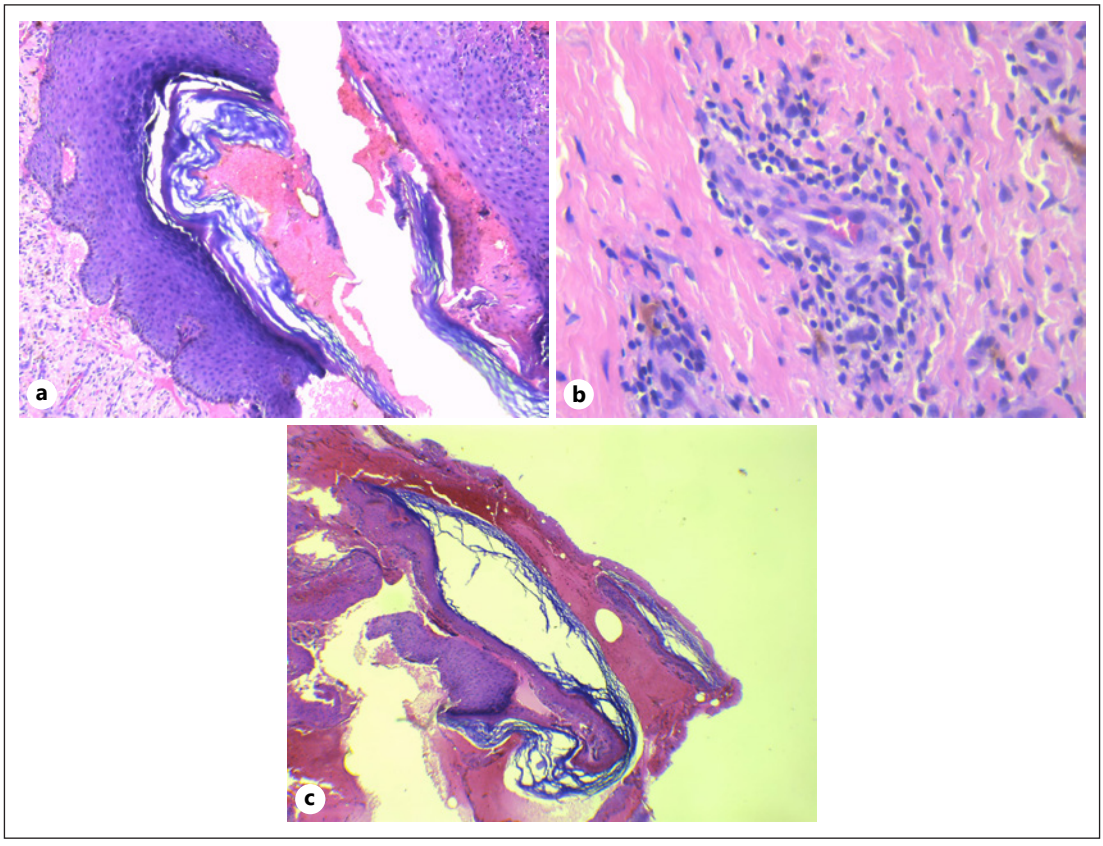


Fig. 5. a–c Histopathological image of anatomical pathology analysis taken from the EB lesions. HE stain, ×100 magnification.

areas were covered with crusts (Fig. 5a). Bleeding and lymphocyte inflammatory cells were found on the tissue biopsy, along with histiocytes and neutrophils (Fig. 5b). We also found subepidermal cleft on other parts of the biopsy (Fig. 5c). The dermis consisted of hyperemic fibro-collagenous tissue with lymphocytes and histiocytes. There were no malignancy signs found in the biopsy sample. The result of the histology examination of the biopsy was consistent with EB. PAS stain demonstrates the level of the split within the basal membrane with most in the subepidermal split (Fig. 6, ×400). This patient has subepidermal bullae with fibrin, scanty inflammatory cells, intact subepidermal blister split, and variable inflammatory infiltrate in the dermis (Fig. 6a). Figure 6b shows subepidermal bulla in ×400 magnification.

Laboratory Analysis

Vitamin D level analysis was performed for the patient and her mother. We did not find any abnormalities in terms of albumin, blood glucose, urea, creatinine, urea nitrogen, and eGFR but did find a deficiency of vitamin D 25-OH (8.6 ng/mL; reference level: 30–100 ng/mL), while the serum phosphate of the patient was in the normal range (3.8 mg/dL; reference level: 2.7–4.5 mg/dL).

Her mother also has an insufficiency of vitamin D 25-OH (23.4 ng/m; normal levels: 30–100 ng/mL). These results support the diagnosis of rickets. However, we are not sure whether the low level of vitamin D in her mother is also because of limited sun exposure.

Case Rep Dermatol 2022;14:283–293
DOI: 10.1159/000525068

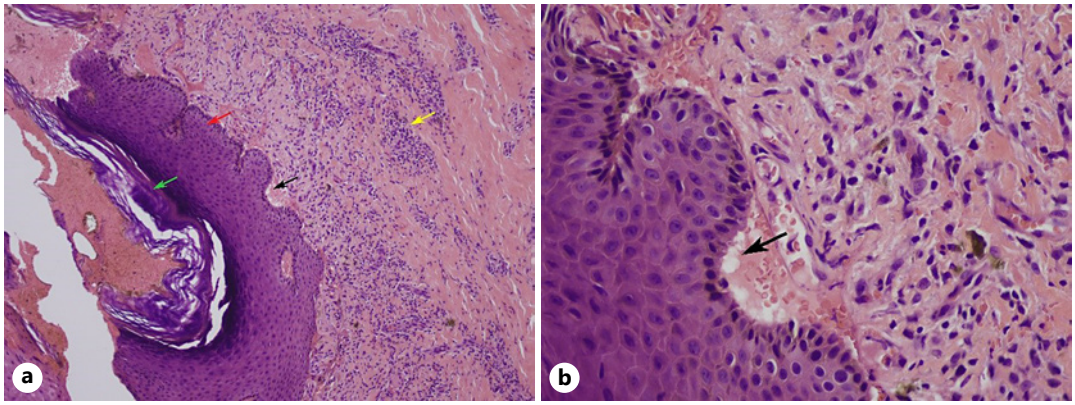


Fig. 6. a, **b** Hallmark features of EB were found in this patient. Black arrow: subepidermal bulla; yellow arrow: dermis stromal with scattered lymphocyte and histiocyte; red arrow: epidermis; green arrow: hyperkeratosis. PAS stain, ×400 magnification.

Molecular Analysis

Exome sequencing was conducted at the Genome Diagnostics laboratory in Radboud UMC, Nijmegen, The Netherlands, after exome enrichment (Agilent SureSelectQXT Human All Exon) on the Illumina NovaSeq Platform (Illumina, San Diego, CA, USA). Variant annotation, selection, and prioritizing for pathogenicity were conducted using their in-house developed strategy. Exome sequencing did not reveal a pathogenic variant in the EB and osteogenesis imperfecta (OI) genes, nor in *PHEX*, the gene for X-linked dominant rickets. The ALPL gene for hypophosphatemia also did not have a pathogenic variant.

Therapy

This patient has been administered 5,000 IU vitamin D once daily and ceramide-based moisturizing cream for 6 months. After vitamin D therapy was given, the patient experienced fewer skin blistering lesions, and no new lesions appeared.

Discussion/Conclusion

EB simplex itself is an inherited disorder characterized by the presence of disturbed skin layer cornification and cell fragility [7]. Many forms of EB exist, and many genes are involved. In the most common forms of EB, the pathological basis is mutations in the genes that encode the epidermal keratins or collagen type VII, which is involved in the attachment between the layers of the dermis [8]. Pathogenic variants of the K5/K14 genes, which are highly expressed in basal keratinocytes, will result in the occurrence of intraepidermal blistering and cell lysis of the basal layer of the skin, a hallmark feature of EB [9]. Mutations in *COL7A1* cause dystrophic EB, which can be either recessive and severe or dominant and mild. *COL7A1* contains 118 exons that encode the alpha-1 chain of collagen type VII, which consists of 2,944 amino acids. This collagen functions as an anchor between the epithelial tissue and the stroma underneath [10].

The findings in our patient are similar to a previous case presented by Roy et al. [11] from India, which presented with epidermolysis hyperkeratosis with vitamin D deficiency rickets. Our patient had similar vesico-bullous lesions and rickets signs and symptoms as the patient presented in the former case report. However, the patient in the former case

Case Rep Dermatol 2022;14:283–293
DOI: 10.1159/000525068

report was born from a consanguineous marriage, which is known to increase the possibility of recessive genetic abnormalities such as EB [11]. Rickets, however, can follow an X-linked dominant pattern of inheritance. We hypothesized that there could be a deletion of two proximal genes, one involved in EB and one in OI; however, no pathogenic variants that cause EB or OI were found by exome sequencing. However, whole-exome sequencing (WES) is not really suitable for the detection of copy number variations (CNVs) due to the relatively low reading depth of many exons. WES itself is a method that allows the sequencing of all protein-coding regions of genes in a genome. Consequently, heterozygous CNV variants may have been missed [12].

An extremely low level of vitamin D was found in the patient and deficiency in the mother, suggesting that the severe vitamin D deficiency of the patient may be the cause of her severe rickets. Vitamin D deficiency is commonly found in EB, with a prevalence ranging from 67% to 76% of patients with EB [1]. Vitamin D deficiency is one of the most common etiologies for rickets. Rickets itself has several hallmark features, such as progressive bowing deformity of the leg bones, disturbed walking gait (waddling gait), abnormal knock-knee deformity (with an intermalleolar distance of >5 cm), swelling of the wrists, and costochondral junction (also known as "rachitic rosary"), and prolonged (>3 months) bone pain [4]. These features can be found in our patient, who recently had severe bone and joint pain, progressive bone bowing deformity, and disturbed waddling gait.

Rickets is associated with vitamin D deficiency due to minimal sunlight exposure. A study has shown that toddlers who experienced a lack of sunlight exposure had more often low vitamin D levels and showed a higher prevalence of rickets [13]. Adequate vitamin D synthesis can be obtained by exposure to the midday sun (from 10 a.m. to 3 p.m.) for 10–15 min per day [14]. Inadequate exposure to sunlight will cause the reduced synthesis of vitamin D in the skin. In females, it is often exacerbated by the use of whole-body clothing, especially in Muslim and Hinduism communities, preventing adequate exposure to sunlight through the use of thick sunblock, enclosed clothing that covers the majority of the skin [5]. Socioeconomic problems may also prevent adequate intake of vitamin D-containing foods, as most of the vitamin-containing foods (such as cow's milk, cod liver oil, and fortified infant formula) are rather unaffordable, especially for families coming from lower socioeconomic levels [5, 15].

The treatment for vitamin D deficiency rickets is the supplementation of vitamin D in the form of ergocalciferol or cholecalciferol [5]. Patients should be advised to take 5,000 IU vitamin D supplementation daily to prevent the reoccurrence of vitamin D deficiency [4]. In our patient, after vitamin D therapy was given, the amount of squama in the patient decreased, and no new lesions appeared. For bone deformities, orthopedic intervention is required in cases of genu valgum and genu varum, especially if the bone deformities cause an ambulatory disturbance [4]. The phosphate level in our patient was normal; however, low phosphate can aggravate bone loss in patients with EB due to hypophosphatasia. Hypophosphatasia itself is a metabolic bone disease due to loss of function mutations in the *ALPL* gene that encodes the cell-surface tissue-nonspecific isoenzyme of alkaline phosphatase (TNSALP) [16].

Based on the findings in our patient, we considered that there is a correlation between EB and low levels of vitamin D, which results in the occurrence of rickets. However further additional cases are needed to prove this finding. The serum phosphate level was normal; therefore, we concluded that the patient does not have hypophosphatemic rickets and that there is no sign of increased bone turnover. Further studies should be conducted to detect possible gene abnormalities related to EB and rickets in this patient that may have been missed by the WES technique, such as CNVs and translocations.

Case Rep Dermatol 2022;14:283–293
DOI: 10.1159/000525068

Acknowledgments

We would like to thank Erik-Jan Kamsteeg, Ph.D., and Helger Yntema, Ph.D., from the Genome Diagnostics Radboud University Medical Center, Nijmegen, The Netherlands, for their contribution to the molecular diagnostic analysis.

Statement of Ethics

Written informed consent was obtained from the patient for the publication of this case report, including the publication of accompanying images (clinical pictures and examination results). We did not censor the images of the patient's face as there are some abnormalities present around the eye region that might be obscured if image censorship was done to the photos. This study has been ethically approved by Prof. Banundari Rahmawati, the head of the Medical Research Ethics Commission of the Diponegoro University/Kariadi General Hospital Semarang with ethics approval certificate number 890/EC/KEPK-RSDK/2021.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This research is fully funded by the Diponegoro University WCRU Grant No. 118-03/UN7.6.1/PP/2021 for laboratory examination and treatment. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. SMHF received funding from the Institute for Research and Community Services (LPPM), Diponegoro University.

Author Contributions

Renni Yuniati performed clinical analysis and drafted the manuscript. Rakhma Yanti Hellmi performed clinical diagnosis. Gema Citra Dwijayanti collected clinical data and reviewed the manuscript. Meira Dewi Kusuma Astuti, Gerard Pals, and Dimitra Micha performed molecular analysis and reviewed the manuscript. Sultana MH Faradz designed the study, performed molecular diagnosis, and reviewed the manuscript.

Data Availability Statement

All data generated or analyzed in this study are included in this article. Further inquiries can be directed to the corresponding author.

References

1 Reimer A, Hess M, Schwieger-Briel A, Kiritsi D, Schauer F, Schumann H, et al. Natural history of growth and anaemia in children with epidermolysis bullosa: a retrospective cohort study. Br J Dermatol. 2020 Jun 1;182(6):1437–48.

Case Rep Dermatol 2022;14:283–293
DOI: 10.1159/000525068

2 Bardhan A, Bruckner-Tuderman L, Chapple ILC, Fine JD, Harper N, Has C, et al. Epidermolysis bullosa. Nat Rev Dis Prim. 2020 Dec 1;6(1):78–27.
3 Hwang SJE, Daniel BS, Fergie B, Davey J, Murrell DF. Prevalence of anemia in patients with epidermolysis bullosa registered in Australia. Int J Womens Dermatol. 2015;1(1):37–40.
4 Allgrove J, Shaw NJ. A practical approach to vitamin D deficiency and rickets. Endocr Dev. 2015;28:119–33.
5 Sahay M, Sahay R. Rickets-vitamin D deficiency and dependency. Indian J Endocrinol Metab. 2012;16(2):164–76.
6 Tukaj S, Bieber K, Witte M, Ghorbanalipoor S, Schmidt E, Zillikens D, et al. Calcitriol treatment ameliorates inflammation and blistering in mouse models of epidermolysis Bullosa acquisita. J Invest Dermatol. 2018;138(2):301–9.
7 Boeira VL, Souza ES, Rocha Bde O, Oliveira PD, Oliveira MF, Rêgo VR, et al. Inherited epidermolysis bullosa: clinical and therapeutic aspects: epidermólise bolhosa hereditária: aspectos clínicos e terapêuticos. An Bras Dermatol. 2013;88(2):185–98.
8 Mariath LM, Santin JT, Schuler-Faccini L, Kiszewski AE. Inherited epidermolysis bullosa: update on the clinical and genetic aspects. An Bras Dermatol. 2020 Sep–Oct;95(5):551–69.
9 Sprecher E, Yosipovitch G, Bergman R, Ciubutaro D, Indelman M, Pfendner E, et al. Epidermolytic hyperkeratosis and epidermolysis bullosa simplex caused by frameshift mutations altering the V2 tail domains of keratin 1 and keratin 5. J Invest Dermatol. 2003 Apr 1;120(4):623–6.
10 Yang N, Ma Y, Yao H, Chang Q, Zhang V, Liang Z, et al. Novel biallelic variants in COL7A1 cause recessive dystrophic epidermolysis bullosa. Mol Genet Genomic Med. 2020 Aug 1;8(8):e1347.
11 Roy A, Sarkar D, Dey P, Pal S. Epidermolytic hyperkeratosis with Vitamin D deficiency rickets in a 5-year-old Indian girl. Indian J Paediatr Dermatol. 2015;16(2):99.
12 Zhao L, Liu H, Yuan X, Gao K, Duan J. Comparative study of whole exome sequencing-based copy number variation detection tools. BMC Bioinformatics. 2020 Mar 5;21(1):97–10.
13 Ekbote VH, Khadilkar AV, Mughal MZ, Hanumante N, Sanwalka N, Khadilkar VV, et al. Sunlight exposure and development of rickets in Indian toddlers. Indian J Pediatr. 2010 Jan;77(1):61–5.
14 Wacker M, Holick MF. Sunlight and vitamin D. Dermatoendocrinol. 2013 Jan 1;5(1):51–108.
15 Liyanage G, de Silva Y. Vitamin D deficiency rickets and COVID-19 pandemic. Case Rep Pediatr. 2021 Apr 15;2021:5512668.
16 Lin EL, Gottesman GS, McAlister WH, Bijanki VN, Mack KE, Griffin DM, et al. Healing of vitamin D deficiency rickets complicating hypophosphatasia suggests a role beyond circulating mineral sufficiency for vitamin D in musculoskeletal health. Bone. 2020 Jul 1;136:115322.