Serum matrix metalloproteinases in patients with different types of cutis laxa: a case series

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Cutis laxa (CL) is a connective tissue disease that is either inherited or acquired. It is characterized by redundant, pendulous, and inelastic skin. Loss of elasticity is a pathological feature of some degenerative and inflammatory diseases. Matrix metalloproteinases (MMPs) can cleave elastin fibers by damaging the microfibrils and the elastin core, resulting in the loss of elasticity. In this study, we report eight patients with different types of cutis laxa along with the quantitative measurement of serum levels of MMP-2 and MMP-9. The cutis laxa patients showed various clinical and histopathological findings, indicating the heterogeneity of this rare skin connective tissue disease. The serum level of MMP-2 and MMP-9 were elevated in these patients. Increased MMP-2 and MMP-9 might be associated with cutis laxa. However, our findings need to be validated in larger clinical settings.

Keywords: cutis laxa, matrix metalloproteinases, elastin, collagen

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INTRODUCTION

Cutis laxa is a rare cutaneous syndrome belonging to a group of heterogeneous disorders. The disease manifests as wrinkled and redundant skin. In addition, excessive skin folds represent the histological hallmark of cutis laxa [1]. The condition can be classified as acquired cutis laxa or inherited cutis laxa, but the clinical presentations overlap between the different types [1].

Acquired cutis laxa comprises a monogenic defect that starts with inflammatory skin eruptions that induce abnormal elastin metabolism, proneness to elastic degradation, and skin wrinkling [2]. Elastolysis may spread to internal organs, giving rise to emphysema and aortic root alteration [1]. Inherited cutis laxa syndrome can be classified as autosomal dominant, autosomal recessive, and X-linked recessive cutis laxa, resulting from monogenic defects that impair elastic fiber assembly [3]. Moreover, several forms of cutis laxa remain unclassified.

Autosomal dominant cutis laxa is a genetically heterogeneous disorder regarded as a connective

tissue disorder characterized by wrinkled and sagging inelastic skin along with some internal organ involvement [4,5]. Autosomal recessive type 2 cutis laxa (ARCL2A and ARCL2B) is a more benign condition of genetically heterogeneous disorder associated with growth, developmental, and skeletal anomalies [6].

The diagnosis of cutis laxa is primarily based on clinical evaluation, which is supported by histopathological and molecular analysis [1]. Microscopic observation of cutis laxa skin biopsies generally reveals a reduction in elastic fibers with fragmentation [4]. These findings can help differentiate cutis laxa from other skin connective tissue disorders, including Ehlers-Danlos syndrome, pseudoxanthoma elasticum, and Marfan syndrome. However, clinical and molecular heterogeneity associated with cutis laxa might result in some difficulties in the diagnosis.

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that consist of several proteins, enabling the degradation of almost all components in the cutaneous extracellular matrix, especially structural collagen molecules and elastic fibers [7]. Gelatinase B (MMP-9), among the subclasses of MMPs, is required for the degradation of collagen IV and degenerated collagen I [8]. MMP-12 (macrophage metalloelastase) has been associated with the hydrolysis of elastic tissues in anetoderma and macrophage migration in cutaneous granulomas [9]. Some studies indicate the alteration of collagenase activity in cutis laxa, suggesting a possible role in its pathogenesis [8,9]. In this study, we report eight patients with different types of cutis laxa along with the quantitative measurement of their serum MMP- 2 and MMP-9 levels.

PATIENTS AND METHODS

This study recruited eight patients with cutis laxa at Loghman Hospital, Tehran, Iran. The Ethics Committee of our center approved the study (Code: 4-519-24.07.90). All the patients provided written informed consent, and the study was performed as per the principles outlined in the Declaration of Helsinki. Enrolled patients were completely examined, and photographs were taken using an SLR camera (Canon EOS-40D). Skin biopsies were taken and sent to the laboratory for histopathology examination under the light microscope, electronic microscope, and direct immunofluorescence.

Genetic analysis was performed on some patients with inherited cutis laxa (patients 2, 4, and 5). According to the standard salting-out method, genomic DNA was isolated from the patients' tissues. Sanger sequencing of *ELN*, *FBLN4*, *FBLN5*, *LTBP4*, *ATP6V0A2*, *PYCR1*, *ALDH18A1*, *ATP7A*, and *COG7* were performed in these patients.

Furthermore, 8 ml of blood was taken from each patient and centrifuged. The serums were stored at -70 °C for less than one month. Subsequently, MMP-2 and MMP-9 were assayed with a commercial ELISA kit (Ray Biotech, Norcross, GA, USA). The normal range of serum MMP-2 was 70-93 ng/ml, while the normal range of serum MMP-9 was 84-103 pg/ml.

CASE DESCRIPTION

Inherited cases of cutis laxa

Patient 1

The patient was a 16-year-old girl with a two-year history of severe skin laxity on the upper limbs and the trunk, though the face was spared (Figure 1a). Mild mitral and tricuspid regurgitation were detected on echocardiography. A hiatal hernia was also demonstrated on a barium swallow. Osteoporosis was conspicuous. The patient was mildly mentally disabled, but neuromotor development was normal. No musculoskeletal or internal organ abnormality was found.

The histopathology examination revealed severe elastorrhexis (fragmentation of elastin) and a sharp decrease in elastin fibers in the dermis (Figure 2a). Electron microscopy revealed disorganized and thick collagen fibers and amorphous materials around degenerated elastic fibers (Figure 3a). The result of direct immunofluorescence was negative.

Patient 2

The patient was a one-month-old neonate girl with severe laxity of the skin and joints. On the physical examination, the skin was completely lax and had lost its elasticity. She had facial dysplasia with special features, including micro-retrognathia, a flat midface, frontal bossing, wide fontanels, a wide nasal bridge, and hypertelorism (Figure 1b). Her parents were cousins. She was the second child of the family. The patient's brother had died at six

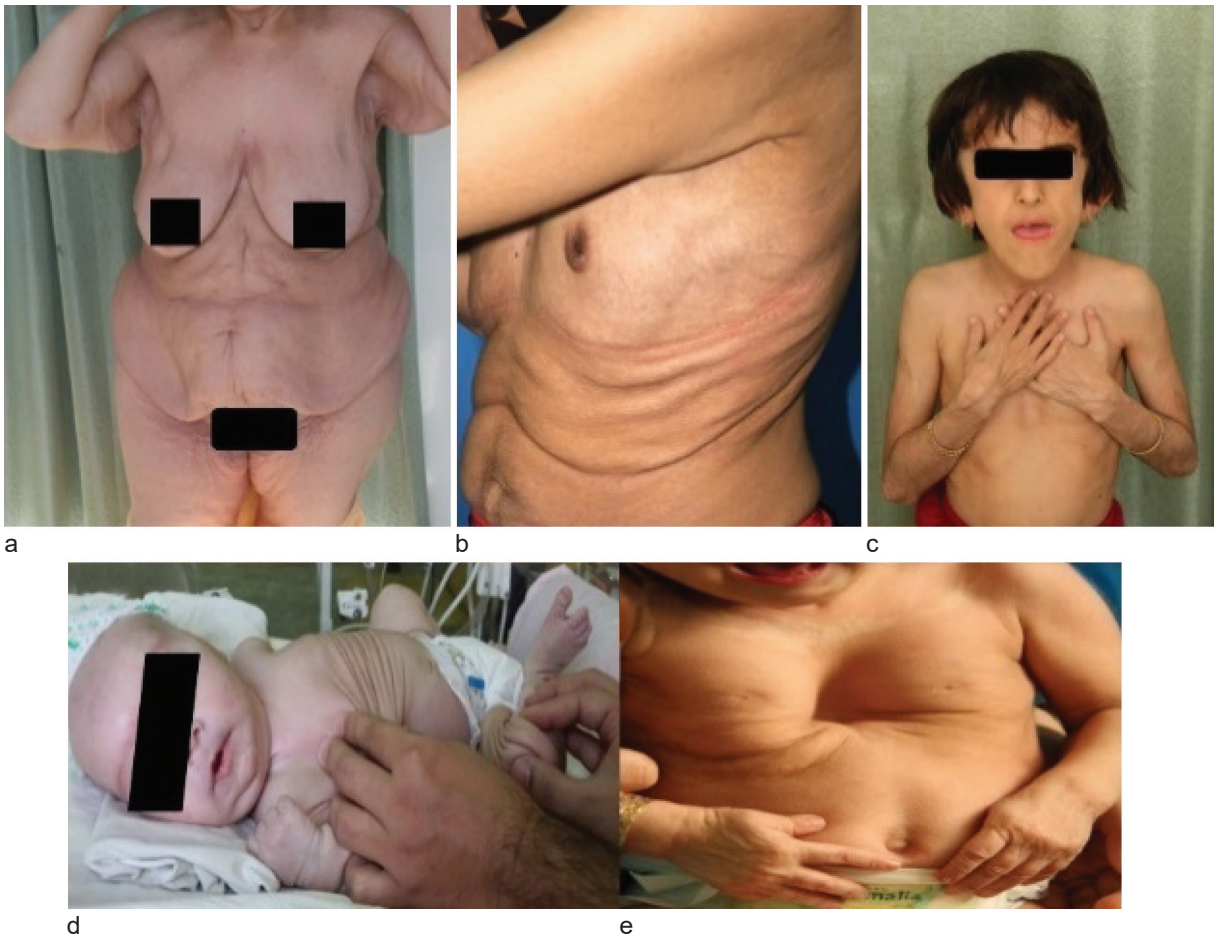


Figure 1. Clinical photographs of the patients with inherited cutis laxa: (a) Skin laxity on upper limbs and the trunk in a 16-year-old girl (Patient 1); (b) Severe laxity of skin and joints in a one-month-old neonate girl with facial dysplasia (Patient 2); (c) Pedunculated and lax skin of the trunk (Patient 3); (d) Increased skin laxity with a pinched nose, triangular and pulled face, and pectus excavatum in a two-year-old girl (Patient 4); (e) Pinched nose, triangular face, drawing chin, lax skin, and prominent skin vessels in a seven-year-old girl (Patient 5)

months because of a similar disease.

The echocardiography assessment detected moderate tricuspid regurgitation and a patent foramen oval. Multiple diverticula (tortuosity) were also found in the intestinal wall, bladder, and urethra. The patient was hospitalized several times because of severe respiratory distress and tracheomalacia. Unfortunately, the patient died at six months, similar to her brother. The patient's frozen samples were sent for genetic analysis. According to genetic analysis, a homozygous likely pathogenic variant of latent transforming growth factor binding protein (LTBP4) was detected. The variants in LTBP4 are associated with autosomal recessive type cutis laxa, also known as Urban-Rifkin-Davis Syndrome.

Patient 3

The patient was a 27-year-old girl. The skin was lax and pedunculated over the extensor surfaces of the limbs, abdomen, and thighs (Figure 1c). The face was not involved. Coxa varus and mild intellectual disability were also observed. Histopathologic examination showed a severe decrease in elastin fibers along with a critical decrease in collagen fibers (Figure 2b). In addition, elastin fibers were damaged by electron microscopy examination (Figure 3b). Unfortunately, the patient refused any further evaluation.

Patient 4

The patient was a two-year-old girl, and her parents were relatives. The pregnancy was

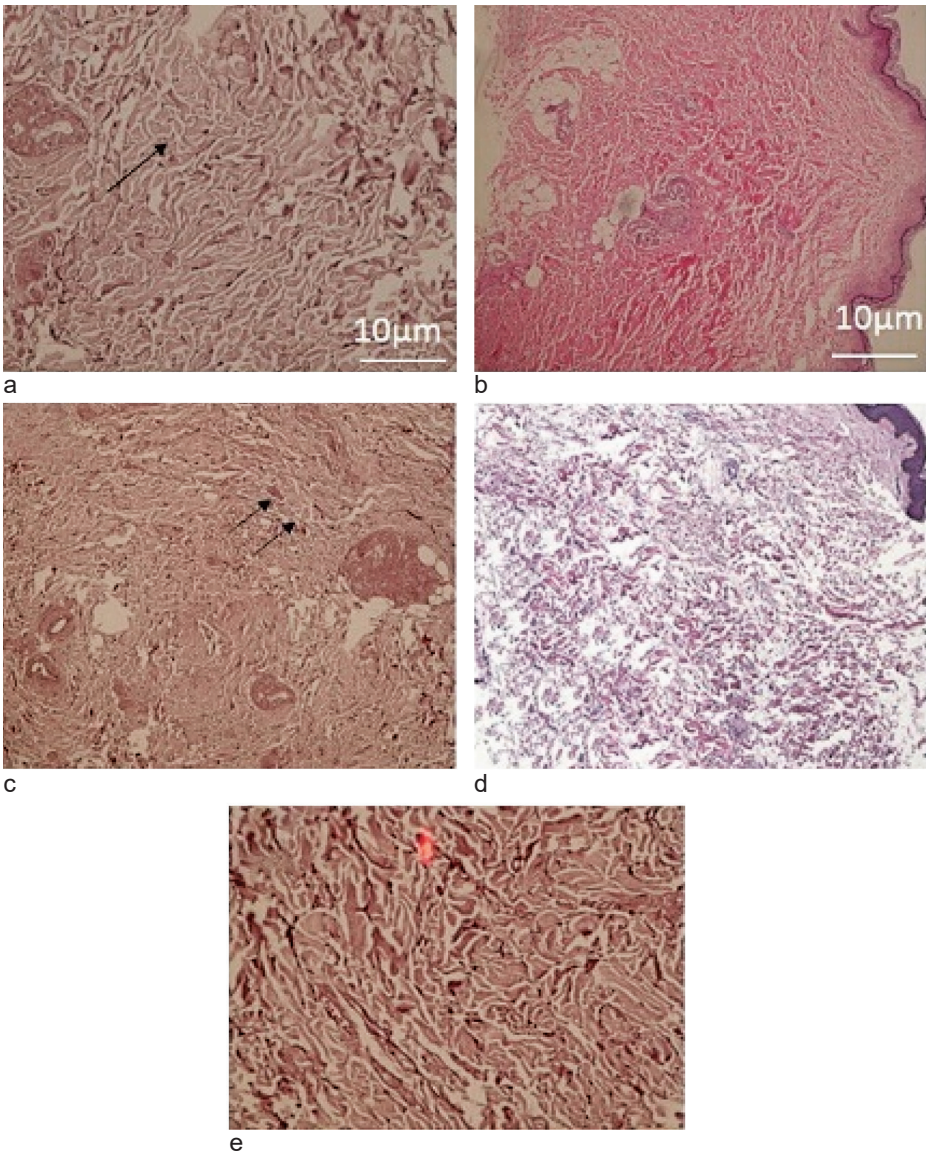


Figure 2. Histopathological H&E staining revealed: (a) Severe elastorrhexis with a sharp decrease in elastin fibers in the dermis (H&E ×40) (Patient 1); (b) A sharp decrease in elastin and collagen fibers in the dermis (H&E ×40) (Patient 3); (c) A severe decrease in elastin fibers along with a critical decrease in collagen fibers in the dermis (H&E ×40) (Patient 6); (d) Decreased to complete loss of elastin fibers with some bundles of collagen in the dermis (H&E ×40) (Patient 7); (e) Sparse to nearly absent elastin fibers in the dermis (H&E ×100) (Patient 8)

uncomplicated. Some motor and cognitive disorders were found after birth. Moreover, she had a pinched nose, triangular and pulled face, pectus excavatum, and hyperextensibility of joints, particularly at the wrists, ankles, and toes (Figure 1d). Lax skin, prominent vessels on the chest and abdomen due to the atrophy of the skin and subcutaneous fat, congenital hip dysplasia, knee subluxation, and osteoporosis were also noted. Wrinkles were most prominent on the back of the hands, feet, and abdominal skin. The genetic analysis confirmed the diagnosis of cutis laxa with homozygous variations on the *PYCR1* gene.

Patient 5

The patient was a seven-year-old girl, the second child of the family born to related parents. The patient had a family history of a similar disease, but the

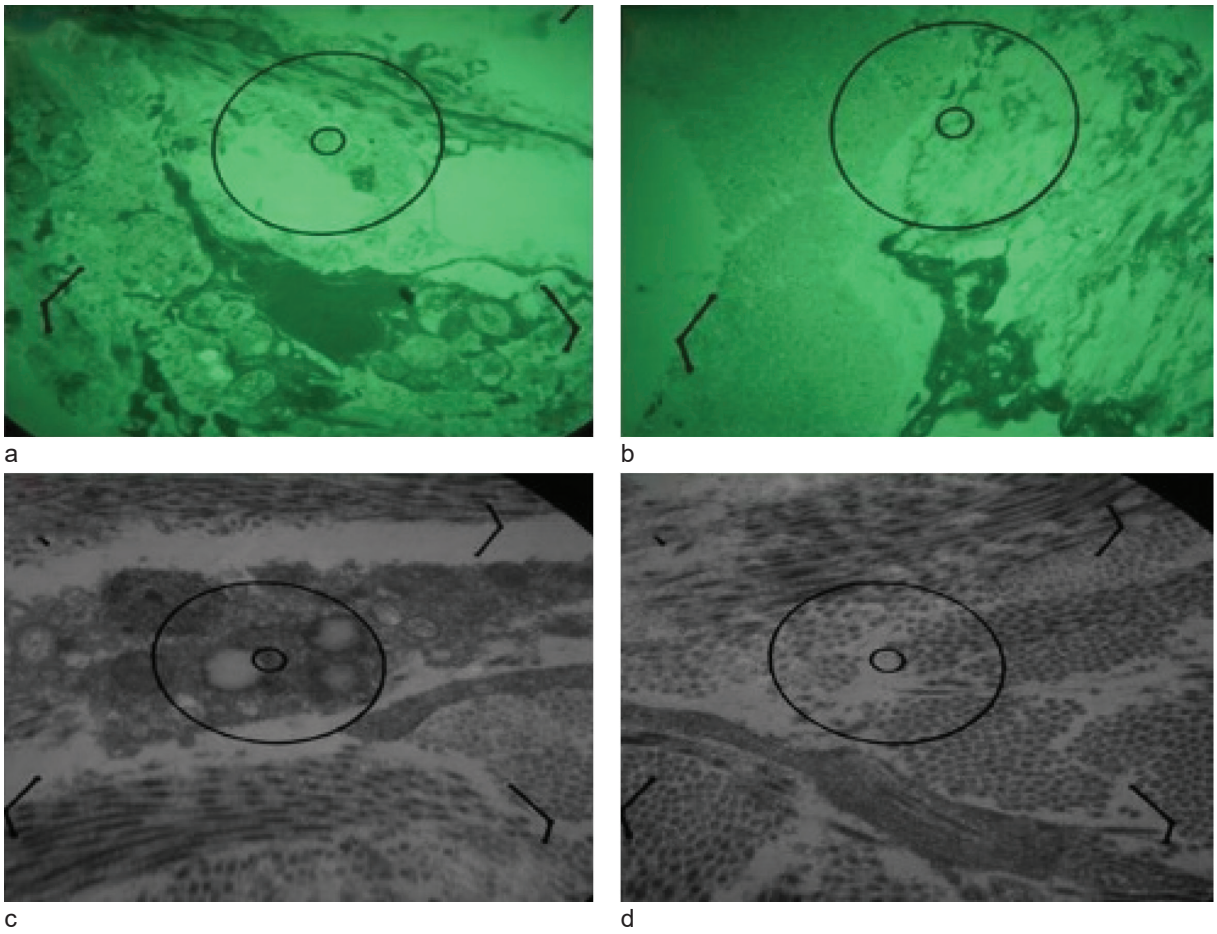


Figure 3. Electron microscopic analysis of the skin: (a) Disorganized and thick collagen fibers and amorphous materials around degenerated elastic fibers (Patient 1); (b) Globules of elastin fiber in the dermis with adjacent bundles of microfibrils (arrows) indicating elastin fibers damage (Patient 3); (c) Disorganized and thick collagen fibers and amorphous materials around degenerated elastic fibers (Patient 6); (d) Damaged elastic fibers with amorphous materials in destructed areas (Patient 8)

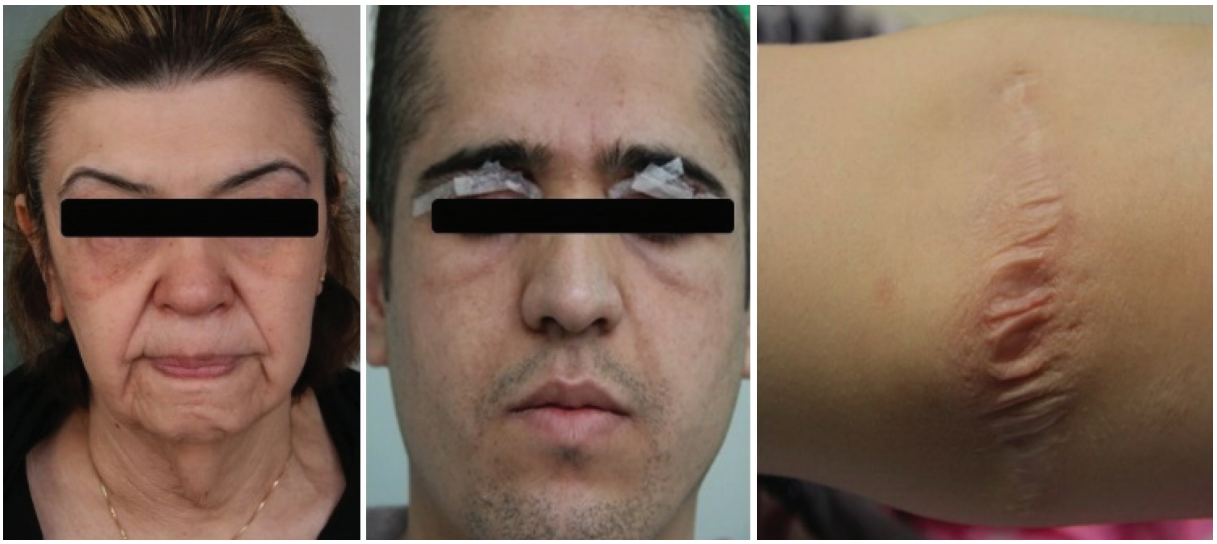
family's first child was normal. Although she initially developed normally, mental retardation was seen later on after birth. Additionally, a pinched nose, triangular face, drawing chin, lax skin (especially on the trunk and limbs), and prominent skin vessels were observed (Figure 1e). Moreover, mild myopia and astigmatism, congenital hip dysplasia, osteoporosis, bone growth retardation, pes planus, pectus excavatum, and sunken eyes were detected. Cutis laxa was confirmed by genetic analysis, which revealed *PYCR1* gene homozygous variations.

Acquired cases of cutis laxa

Patient 6

The first patient was a 40-year-old woman with severe recalcitrant urticaria following the administration of penicillin. Progressive skin laxity occurred in the patient six months after the appearance of urticaria. The disease was insidiously progressive, and the 40-year-old patient looked older than her actual age (Figure 4a). Moreover, membranoproliferative glomerulonephritis (MPGN) and umbilical hernia appeared about one year later. The renal involvement was recalcitrant. In bone densitometry, severe osteoporosis was detected.

Histopathology examination, including orcein staining, showed decreased to complete loss of elastin fibers in the dermis, fragmented epidermis, and epidermal atrophy. The histopathological analysis also displayed collagen bundles in the dermis (Figure 2c). In electron microscopy, scattered, disorganized, and thick collagen fibers were seen in the dermis. Moreover, areas of amorphous



a b c

Figure 4. Clinical photographs of the patients with acquired cutis laxa: (a) Progressive skin laxity in a 40-year-old female adult (Patient 6); (b) Considerable skin laxity on the eyelids a few months after the blepharoplasty (Patient 7); (c) Considerable skin laxity on the scar of a previous skin surge (Patient 8)

materials around degenerated elastic fibers were noticeable (Figure 3c).

Patient 7

The patient was a 26-year-old man with a history of blepharoplasty due to a severe drooping eyelid a few months before. He was referred to us after blepharoplasty surgery, so we could not access his preoperative photos. A few months after blepharoplasty, the patient demonstrated considerable skin laxity on the eyelids, trunk, and extremities (Figure 4b). The patient had no history of drug use or prior urticarial reaction. Histopathological evaluation revealed sparse to nearly absent elastin fibers in the dermis (Figure 2d). These findings could be compatible with the diagnosis of cutis laxa, which manifested first as a localized form by blepharochalasis. The patient refused to cooperate with further evaluations due to his severe depression.

Patient 8

A 23-year-old girl was referred to us with a history of multiple surgeries for repairing her lax pedunculated skin. She showed considerable skin laxity on the extensor surfaces of the joints, with numerous scars from previous skin surgeries (Figure 4c). The face was not involved. The patient had no history of any musculoskeletal disorder. Neuromotor and cognitive development were also normal. On skin histopathology examination, a sharp decrease in elastin and collagen fibers was revealed in the dermis (Figure 2e). In addition, elastin fibers were damaged according to electron microscopy examination (Figure 3d). Unfortunately, the patient refused further evaluations.

Serum MMP-2 and MMP-9 measurement

In this case series, serum MMP-2 and MMP-9 levels were assessed in all eight patients. The results showed that the MMP-2 and MMP-9 serum levels were significantly higher than the normal ranges of 70-93 ng/ml and 84-103 pg/ml, respectively (Table 1).

Table 1. The patients' serum MMP-9 and MMP-2 levels

	P1	P2	P3	P4	P5	P6	P7	P8
MMP-9 (pg/ml)	310,400	184,200	3,750	36,950	127,400	67,000	70,000	250
MMP-2 (ng/ml)	16,426	1,350	5,734	3,986	4,410	1,988	3,694	7,242

P = patient; MMP = matrix metalloproteinase

DISCUSSION

Cutis laxa is characterized by abnormal elastic fibers resulting in loose, redundant, hypoelastic skin. The skin can easily be pulled away from the underlying tissue and slowly returns to its original position. These findings are often most noticeable on the neck, hands, and groin, but can also be seen on the face, creating a premature aging appearance. Cutis laxa is not characterized by easy bruising or abnormal scarring in comparison with some other skin connective tissue diseases. Cutis laxa may be related to autoimmune disease and can also be inherited or acquired. Inherited forms include autosomal dominant cutis laxa, autosomal recessive cutis laxa, Urban-Rifkin-Davis syndrome, macrocephaly-alopecia-cutis laxa-scoliosis syndrome, and arterial tortuosity syndrome (ATS) or X-linked cutis laxa [10]. Herein, we have reported both inherited and acquired forms of cutis laxa with their various clinical and histopathological characteristics. However, there are significant overlaps among different types of cutis laxa, and definite clinical classification can be difficult. The acquired form is rare and has been associated with different conditions, such as heavy chain deposition disease [11].

Previous studies have demonstrated an imbalance between proteases such as trypsin, cathepsin, and matrix metalloproteinases (MMPs) and their inhibitors, which can result in abnormal elastin and collagen catabolic processes. MPPs are involved in tissue remodeling, cell migration, angiogenesis, and tumor cell metastasis [10,12]. Moreover, MMPs are responsible for the breakdown of collagen in the skin of cutis laxa patients; an increased level of collagenase activity has been shown in fibroblasts derived from patients' skin [10]. The study by Hatamochi *et al.* indicated elevated expression levels of MMP-1, MMP-3, and MMP-9 in the fibroblasts of cutis laxa patients. They concluded that increased expression of MMPs may be associated with histopathological abnormality in cutis laxa patients [13]. In another study, levels of expression of MMP-3, MMP-7, and MMP-9 were significantly increased in the culture media of anetodermic skin compared to uninvolved skin. They suggested that these metalloproteinases could be involved in the degradation of elastic fibers in anetodermic skin [14]. Thus, MMP expression may provide considerable insight into the pathogenesis of cutis laxa. In the current study, we investigated the serum levels of MMP2 and MMP9 in eight patients with cutis laxa. Most of our patients revealed loss of elastin fibers in their histopathological analysis. In addition, elevated MMP-2 and MMP-9 were observed in all eight cases, which agreed with previous studies [10,15].

In our study, patients 1 and 2 revealed the highest serum levels of MMP-9, and we found severe skin laxity in patient 1. Moreover, patient 2 died of systemic complications of cutis laxa. Moreover, the severe skin laxity in patient 1 displayed a positive correlation with the highest serum levels of both MMP-2 and MMP-9. Regarding the lowest serum levels of MMP-9 in patients 3 and 8 associated with the mildest type of cutis laxa, we suggest that the elevated level of MMP-9 may be correlated with the disease severity. Therefore, sharply elevated MMP-9 and MMP-2 may lead to severe cutis laxa and elastin degradation.

Our study had limitations, such as limited sample size and lack of any control group due to the rarity of this skin disease and our restricted facilities. However, this report could be a completely distinctive pilot study about eight patients with different types of cutis laxa as a rare skin condition, along with the measurement of their serum levels of MMPs. The results suggest the possible role of MMP-2 and MMP-9 in the pathogenesis of cutis laxa. Surely, future multi-center studies with a more structured and controlled design could be beneficial to elucidate any possible role of MMPs in the pathogenesis of cutis laxa.

Author contributions

AE: Conceptualization, study supervision, and study validation

FM: Study supervision, conceptualization, and study validation

MK: Data gathering and electron microscopic evaluation

AK: Data gathering and writing the manuscript

FS: Data interpretation and writing the manuscript

RR: Data interpretation, writing, reviewing, and revising the manuscript

All authors have read and approved the final manuscript.

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Consent for publication

The patients or patient guardians provided written informed consent to the publication of their case details.

Conflict of interest: None declared.

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