Serum matrix metalloproteinases in patients with different types of cutis laxa

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Abstract

Cutis laxa is a connective tissue disease, which is either inherited or acquired with various clinical presentation. Increased level of MMP-2 and MMP-9 might be associated with the cutis laxa disease. However, our findings in current experience need to be validated in larger clinical settings.

Introduction

Cutis laxa (CL) is a rare inherited syndrome, which are belonged to a group of heterogeneous disorders. The clinical characteristic of the disease manifested as wrinkled and redundant skin. In addition, excessive skin folds represents the histological hallmark of cutis laxa (1). Cutis laxa can be classified as acquired cutis laxa (ACL) and inherited cutis laxa but the clinical presentation displayed extensive overlap between the different phenotype (1).

Acquired disorder of cutis laxa is comprises of monogenic defect, starts with skin eruption inflammatory that induce an abnormal elastin metabolism, proneness to elastic degradation, and skin wrinkling (2). Elastolysis may spread to internal organs giving rise to emphysema and aortic root (1). Inherited cutis laxa syndrome can be classified as autosomal dominant, autosomal recessive, and X-linked recessive cutis laxa, which resulted 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 that is regarded as connective tissue disorder, characterized by wrinkled and sagging inelastic skin related to internal organ involvement (4, 5). However, autosomal recessive, type 2 (ARCL2A and ARCL2B) is a more benign condition of genetically heterogeneous disorder associated with growth and developmental delay and skeletal anomalies in patients with CL (6). The diagnosis is perfume primarily at clinical validity, which is supported by molecular analysis (1). In general, microscopic observation of CL skin biopsies displayed reduction in elastic fibers with fragmentation (4).Furthermore, clinical and molecular heterogeneity associated with CL might result in some difficulties in its diagnosis.

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that consist of several proteins, enabling degrading almost all components in the cutaneous extracellular matrix (ECM), especially structural molecules of collagen and elastic fibers (7). Gelatinase B (MMP-9) among subclasses of MMPs are required for the degradation of collagen IV and degenerated collagen I (8). MMP-12 (macrophage

metalloelastase) has been associated with the hydrolysis of elastic tissue in anetoderma and macrophage migration in cutaneous granulomas (9). Some studies indicates the alteration of collagenase activity in CL, suggesting a possible role in the pathogenesis of CL (8, 9). In this study, we reported eight patients with different types of CL along with the quantitative measurement of their serum MMP- 2 and MMP-9.

Patients and Methods

In this study, eight patients with cutis laxa recruited to Loghman hospital, Tehran, Iran. The ethics committee of our center approved the study. All the patients gave written informed consent and the study was performed regarding 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 test (DIF).

Furthermore, 8 ml of blood were taken from each patient and were centrifuged. The serums were stored at -70 °C for less than one month. Subsequently, MMP-2 and MMP-9 of these serum samples were assayed with a commercial ELISA kit (Ray Biotech, Norcross, GA, USA). The normal range of MMP-2 serum level was 470-800 ng/ml while the normal range of MMP-9 was 169-705 ng/ml.

Case Description

Inherited case of CL

Patient 1

The patient was a 16-year-old girl with a 2-year history of severe skin laxity on upper limbs and the trunk but the face did not involve (Figure 1A). Mild mitral and tricuspid regurgitation were detected in echocardiography. The hiatal hernia was also demonstrated in barium swallow. Osteoporosis was conspicuous. The patient was mildly mentally retarded but neuromotor development was normal. No musculoskeletal or internal organ abnormality was found.

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

Patient 2

A one-month-old neonate girl with laxity of skin and joints. On the physical examination, the child's skin was completely lax and lost its elasticity. She had facial dysplasia with special features including micro-retrognathia, flat midface, prominent frontal, wide fontanels, 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 6 months because of a similar disease.

In echocardiography assessment, moderate tricuspid regurgitation and patent foramen oval were detected. 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 the age of 6 months similar to her brother. The patient's frozen samples were sent for genetic analysis. According to genetic analyzes, Urban-Rifkin-Davis syndrome (a form of cutis laxa) was diagnosed for the patient and homozygous mutation of latent transforming growth factor binding proteins (LTBP4) on chromosome 19q13 was identified.

Patient 3

The patient was a 27-year-old girl and she was the fifth patient's sister. The skin was lax and pedunculated over the extensor surfaces of the limbs, abdomen (Figure 1C), and thigh. The face was not involved. Coxa varus and mild intellectual disability were also observed in this patient. 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 2-year-old girl that her parents were relative. The pregnancy was uncomplicated. Some motor and cognitive disorders were found after the birth. Moreover, she had a pinched nose, triangular and pulled face, pectus excavatum, hyperextensibility of joints, particularly on 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 found in this patient. Wrinkles were most prominent on the back of the hands, feet, and abdomen skin. The genetic analysis confirmed the diagnosis of CL with homozygous mutations on the PYCR1 gene.

Patient 5

The patient was a 7-year-old girl, the second child of the family who was born from relative parents. The patient had a family history of a similar disease, but the first child of the family was normal. Although she developed normally, mental retardation was seen after the birth. Additionally, pinched nose, triangular face, drawing chin, lax skin, especially on trunk and limbs, and prominent skin vessels have been observed (Figure 1E). Moreover, mild myopia and astigmatism, congenital hip dysplasia, osteoporosis, bone growth retardation, pes planus, pectus excavatum, deep-seated eye (sunken eyes) were detected. Patients had already confirmed having cutis laxa by genetic analysis that proved PYCR1 gene homozygous mutations.

Acquired cases of CL

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 6 months after the appearance of urticaria. The disease was insidiously progressive and the 40-year-old patient looks older than her actual age (Figure 1G). Moreover, membranoproliferative glomerulonephritis (MPGN) and umbilical hernia appeared about one year later. The renal involvement was recalcitrant. In bone densitometry, severe osteoporosis was also detected.

Histopathology examination, including orcein staining, showed decreased to complete loss of elastin fibers in the dermis, epidermis fragmented, 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 materials around degenerated elastic fibers was noticeable (Figure 3C).

Patient 7

The patient was a 26 -year-old man with a history of blepharoplasty due to severe drooping eyelid a few months before. He referred to us after blepharoplasty surgery, so we could not have access to his preoperative photos. A few months after the blepharoplasty, the patient demonstrated considerable skin laxity on the eyelids along with the trunk and extremities (Figure 1H). The patient did not have any 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 belepharochalasis. The patient refused to cooperate for any further evaluations due to his severe depression.

Patient 8

A 23-year-old girl with a history of cutis laxa that was undergone repeated surgery. Multiple surgeries were performed in the past for repairing the lax pedunculated skin. She showed considerable skin laxity on the extensor surfaces of the joints and the scars of the previous skin surgeries (Figure 1I). 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 by electron microscopy examination (Figure 3D). Unfortunately, the patient refused for any further evaluation.

Serum MMP-2 and MMP-9 measurement

In this case series, the assessment of serum MMP-2 and MMP-9 was performed in all 8 patients. The results showed that the MMP-2 and MMP-9 serum levels were significantly higher than the normal ranges of MMP-2 (normal range 470- 800 ng/ml), and MMP-9 (normal range 169-705 ng/ml) (Figure 4).

Discussion

Cutis laxa (CL) is characterized by abnormal elastic fibers resulting in loose, redundant, hypoelastic skin. The skin in CL 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. CL 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 be also inherited or acquired. Inherited forms include autosomal dominant CL; autosomal recessive CL; Urban-Rifkin-Davis syndrome; macrocephaly-alopecia-CL-scoliosis syndrome; and arterial tortuosity syndrome (ATS) or X-linked CL (10). Herein, we have reported both inherited and acquired forms of cutis laxa with their various clinical and histopathological characteristics. However, there is significant overlap among different types of CL 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 that an imbalance between proteases such as trypsin, cathepsin, matrix metalloproteinases (MMPs) and their inhibitors, which can resulted in abnormal elastin and collagen catabolic processes. MPPs are involved in tissue remodeling, cell migration, angiogenesis, and tumor cell metastasis (10, 12). Moreover, matrix metalloproteinases (MMP) are responsible for the breakdown of collage in the skin of CL patients; an increased level of collagenase activity has been shown in fibroblasts derived from the skin of patients (10). The study that has been conducted by Hatamochi et al., indicated elevated levels of expression MMP-1, MMP-3, and MMP-9 in fibroblast of CL patients. They concluded that increased expression of MMPs may be associated with histopathological abnormality in CL 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, MMPs expression may provide considerable insight into the pathogenesis of CL. In the current study, we investigated the serum levels of MMP2 and MMP9 in eight patients with CL. Most of our patients revealed decreased loss of elastin fibers in their histopathological analysis. In addition, elevated MMP-2 and MMP-9 were observed in all eight cases, which was in agreement with the findings of previous studies (10, 15). In our study, patients 2 and 3 revealed the highest serum level of MMP-9 and we found severe skin laxity in patient 2. Moreover, patient 3 unfortunately died of systemic complications of CL. Moreover, the severe skin laxity in patient 2 displayed a positive correlation with the highest serum levels of both MMP-2 and MMP-9. Compared with the lowest serum levels of MMP-9 in patients five and six associated with the mildest type of CL, we suggest that the elevated level of MMP-9 may be correlated with the disease severity. Therefore, the elevated MMP-9 combined with MMP-2 may lead to severe CL and elastin degradation.

However, our study had some limitations such as limited sample size because cutis laxa is a very rare skin disease, and we collected the patients from multiple centers across Iran. Moreover, this report could be a completely distinctive study due to the presentation of eight patients with different types of cutis laxa, a rare skin condition along with the measurement of their serum level of MMP. These results suggest the possible pathogenic role of MMP-2 and MMP-9 in the pathogenesis of cutis laxa.

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their case details.

References

1. Beyens A, Boel A, Symoens S, Callewaert B. Cutis laxa: a comprehensive overview of clinical characteristics and pathophysiology. Clinical Genetics. 2021;99(1):53-66.

2. Hu Q, Reymond J-L, Pinel N, Zabot M-T, Urban Z. Inflammatory destruction of elastic fibers in acquired cutis laxa is associated with missense alleles in the elastin and fibulin-5 genes. Journal of investigative Dermatology. 2006;126(2):283-90.

3. Mohamed M, Kouwenberg D, Gardeitchik T, Kornak U, Wevers RA, Morava E. Metabolic cutis laxa syndromes. Journal of inherited metabolic disease. 2011;34(4):907-16.

4. Callewaert B, Renard M, Hucthagowder V, Albrecht B, Hausser I, Blair E, et al. New insights into the pathogenesis of autosomal-dominant cutis laxa with report of five ELN mutations. Human mutation. 2011;32(4):445-55.

5. Megarbane H, Florence J, Sass JO, Schwonbeck S, Foglio M, De Cid R, et al. An autosomal-recessive form of cutis laxa is due to homozygous elastin mutations, and the phenotype may be modified by a heterozygous fibulin 5 polymorphism. Journal of investigative dermatology. 2009;129(7):1650-5.

6. Noordam C, Funke S, Knoers N, Jira P, Wevers R, Urban Z, et al. Decreased bone density and treatment in patients with autosomal recessive cutis laxa. Acta paediatrica. 2009;98(3):490-4.

7. ASHWORTH JL, MURPHY G, ROCK MJ, SHERRATT MJ, SHAPIRO SD, SHUTTLEWORTH CA, et al. Fibrillin degradation by matrix metalloproteinases: implications for connective tissue remodelling. Biochemical Journal. 1999;340(1):171-81.

8. Fisher B, Costantino J, Redmond C, Poisson R, Bowman D, Couture J, et al. A randomized clinical trial evaluating tamoxifen in the treatment of patients with node-negative breast cancer who have estrogen-receptor–positive tumors. N Engl J Med. 1989;320:479-84.

9. Vaalamo M, Kariniemi A-L, Saarialho-Kere U, Shapiro SD. Enhanced expression of human metalloelastase (MMP-12) in cutaneous granulomas and macrophage migration. Journal of Investigative Dermatology. 1999;112(4):499-505.

10. Gu W, Liu W, Yang X, Yuan X, Tian Y, Meng R, et al. Cutis laxa: analysis of metalloproteinases and extracellular matrix expression by immunohistochemistry and histochemistry. European Journal of Dermatology. 2011;21(5):717-21.

11. New HD, Callen JP. Generalized acquired cutis laxa associated with multiple myeloma with biphenotypic IgG-λ and IgA-ϰ gammopathy following treatment of a nodal plasmacytoma. Archives of dermatology. 2011;147(3):323-8.

12. Suda T, Hara H, Yoshitake M, Ohbayashi T, Nakamura T, Terui T. Immunohistochemical investigation of mid-dermal elastolysis with a history of erythema. The American journal of dermatopathology. 2008;30(5):477-80.

13. Hatamochi A, Kuroda K, Shinkai H, Kohma H, Oishi Y, Inoue S. Regulation of matrix metalloproteinase (MMP) expression in cutis laxa fibroblasts: upregulation of MMP-1, MMP-3 and MMP-9 genes but not of the MMP-2 gene. The British journal of dermatology. 1998;138(5):757-62.

14. Ghomrasseni S, Dridi M, Gogly B, Bonnefoix M, Vabres P, Venencie PY, et al. Anetoderma: an altered balance between metalloproteinases and tissue inhibitors of metalloproteinases. The American journal of dermatopathology. 2002;24(2):118-29.

15. De Almeida Jr HL, Wolter M, De Farias MV, De Castro LAS. Elastic tissue damage in cephalic acquired cutis laxa. Journal of cutaneous pathology. 2008;35(1):58-61.

Figure legend:

Figure 1. Clinical photograph of the patients with Cutis laxa; (A) skin laxity on upper limbs and the trunk in a 16-year-old girl, (B) Severe laxity of skin and joints in a one-month-old neonate girl with facial dysplasia. (C) pedunculated and lax skin of the abdomen, (D) Increased skin laxity with a pinched nose, triangular and pulled face, pectus excavatum in a 2-year-old girl, (E, F) Pinched nose, triangular face, drawing chin, lax skin and prominent skin vessels in a 7-year-old girl. (G) Progressive skin laxity in a 40-year-old female adult, (H) Considerable skin laxity on the eyelids a few months after the blepharoplasty, (I) Considerable skin laxity on the scar of the previous skin surgery.

Figure 2. Histopathological H&E staining revealed; (A) severe elastorrhexis with a sharp decrease in elastin fibers in the dermis (H&E*40), (B) Sharp decrease in elastin and collagen fibers in the dermis (H&E*40), (C) Severe decrease in elastin fibers along with a critical decrease in collagen fibers in the dermisb(H&E*40), (D) Decreased to complete loss of elastin fibers with some bundles of collagen in the dermis(H&E*40), (E) Sparse to nearly absent elastin fibers in the dermis (H&E*100).

Figure 3. Electron microscopic analysis of the skin; (A, patient 1) Disorganized and thick collagen fibers and amorphous materials around degenerated elastic fibers. (B, patient 3) Globules of elastin fiber in the dermis with adjacent bundles of microfibrils (arrows) indicating elastin fibers damage. (C, patient 6) Disorganized and thick collagen fibers and amorphous materials around degenerated elastic fibers. (D, patient 8) Damaged elastic fibers with amorphous materials in destructed areas

Figure 4. Serum levels of MMP-2 and MMP-9 in cutis laxa patients