The impact of COL1A1 and COL6A1 expressions on hypospadias and penile curvature severity

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Abstract

Background: Hypospadias, the most frequent congenital male external genitalia abnormality, is usually associated with curvature of the ventral penile, i.e. chordee. Abnormality of dartos tissue has been suggested as the pathophysiology of the chordee. Collagen is one of the most abundant fibrous proteins within the extracellular matrix. In this study, we determined the expressions of *collagen 1 (COL1A1)* and *COL6A1* in hypospadias patients and associated them with the severity of penile curvature.

Methods: We involved 60 children <18 years old, consisting of 20 distal hypospadias, 20 proximal hypospadias patients, and 20 control in our institution from 2017 – 2020. The expressions of *COL1A1* and *COL6A1* from dartos tissue were determined by reverse-transcriptase polymerase chain reaction (qPCR). The penile curvature severity was classified as mild (<30 degree), moderate (30-60 degree), and severe (>60 degree).

Results: qPCR showed that the *COL1A1* and *COL6A1* expressions were significantly downregulated in distal (0.88 (0.38-2.53) and 0.54 (0.16-4.35), respectively) and proximal 0.76 (0.33-2.57) and 0.57 (0.18-1.38), respectively) hypospadias groups compared to controls (1.85 (0.24-4.61) and 0.93 (0.17-4.06), respectively) with *p*-value of 0.024 and 0.018, respectively. Furthermore, there was a moderate correlation between *COL1A1* and *COL6A1* expressions (r=0.458, p<0.0001). Interestingly, the *COL1A1* and *COL6A1* was also significantly downregulated in the moderate and severe compared to the mild chordee groups with *p*-value of 0.003 and 0.037, respectively.

Conclusions: Aberrant *COL1A1* and *COL6A1* expressions might affect the abnormality of dartos tissue and penile curvature severity in hypospadias patients.

Background

Hypospadias is the most frequent congenital penile anomaly which affects male external genitalia with the incidence of around 1 in 250 male newborns. It is caused by tissue underdevelopment on the ventral aspect of the penis resulting an abnormal location of the urethral opening on the penile underside (1). Elastosonography found deep alteration of hypospadias penile anatomy, in which the corpus spongiosum is stiffer and less elastic with less developed cavernous corpora (2).

Hypospadias is associated with curvature of the ventral penile, called chordee. It is caused by the insufficiency or disorganization of the complex growth (the vascular and fascial structures) of the ventral penile (3). Resection of dartos tissue usually straightens the penis in patients with chordee and buried penis, suggesting the pathophysiology of these anomalies is related to dartos tissue (4).

Extracellular matrix (ECM) is a non-cellular macromolecular network which supports cellular processes, such as proliferation, migration, differentiation, and homeostasis (5). One of the most abundant fibrous proteins within ECM is collagen. It has been considered the main component of the fibrous skeleton of the corpus cavernosum penis and is ubiquitous within the erectile tissues of the human penis (6, 7).

Fibrillar collagens are widely distributed in tissues providing the tensile strength. Most fibrillar collagen is type 1 collagen which can be found throughout the tissue beneath the urethral plate (6, 7). The type 6 collagen (COL6A1) binds to type 1 collagen (COL1A1) to form thicker collagen fibers. COL6A1 is a part of the beaded- filament-forming collagens and the most studied member of its group (8). In this study, we determined the expressions of *collagen 1 (COL1A1)* and *COL6A1* in hypospadias patients and associated them with the severity of penile curvature.

Methods

We involved 60 children < 18 years old, consisting of 20 distal hypospadias, 20 proximal hypospadias patients, and 20 control in our institution from 2017–2020. Detailed history and thorough examination of hypospadias patients were performed by one urologist.

Reverse-transcriptase polymerase chain reaction (qPCR) assays

Fragments of the foreskin were kept in RNAlater (Ambion, AM7021) solution before extraction. This tissue was extracted using Genezol RNA solution (GENEzol™, Cat. No. GZR100). cDNA was synthesized using Excel RT Reverse transcriptase kit (RP1300, SMOBIO, Hsinchu City, Taiwan) with PCR conditions of 25^{O}C for 10 minutes (denaturation), 42^{O}C for 50 minutes (annealing), and 85^{O}C for 5 minutes (extension).

qPCR was done by mixing the cDNA and Taq Master Mix (GoTaq®Green Master Mix, Cat.No. M7122) with these following primer genes: *COL1A1* (F: 5'-TACAGCGTCACTGTCGATGGC-3' and R: 5'-TCAATCACTGTCTTGCCCCAG-3'), *COL6A1* (F: 5'-GACCTCGGACCTGTTGGGTAC-3' and R: 5'-TACCCCATCTCCCCCTTCAC-3') (9), and *GAPDH* (F: 5'-GCACCGTCAAGGCTGAGAAC-3' and R: 5'-TGGTGAAGACGCCAGTGGA-3') (10). qPCR conditions initiated with denaturation at 94 °C for 2 minutes, followed by up to 40 cycles consist of 94 °C for 10 seconds, annealing at 54 °C, 58 °C, 63 °C (*COL6A1, COL1A1*, and *GAPDH*, respectively) for 30 seconds, and 72 °C for 1 minute followed by final extension at 72 °C for 10 minutes. PCR products were then separated by 2% agarose gel along with a 100-bp DNA ladder (Bioron, Germany, Cat. No. 306009) (Fig. 1). Gene expressions was quantified using Image J software for densitometry analysis.

Statistical analysis

Data were analyzed for their normality distribution using Kolmogorov-Smirnov. Kruskal – Wallis and Mann–Whitney *U tests* were used for the data which were not normally distributed while the normally distributed data were analyzed with one-way ANOVA test. $p < 0.05$ was considered as statistically significant.

Results

The baseline characteristics of our patients was described in Table 1. Most patients showed midshaft and penoscrotal hypospadias (67.5%) (Table 1). qPCR showed that the *COL1A1* and *COL6A1* expressions were significantly downregulated in distal (0.88 (0.38–2.53) and 0.54 (0.16–4.35), respectively) and proximal (0.76 (0.33–2.57) and 0.57 (0.18–1.38), respectively) hypospadias groups compared to controls (1.85 (0.24–4.61) and 0.93 (0.17–4.06), respectively) with *p*-value of 0.024 and 0.018, respectively (Table 2). Interestingly, the *COL1A1* and *COL6A1* was also significantly downregulated in the moderate and severe compared to the mild chordee groups with *p*-value of 0.003 and 0.037, respectively (Table 2).

Table 1
Characteristics of all patients in our institution.

Characteristics	**Mean ± SD; N (%)**
Age (years)	7.85 ± 3.9
√ Hypospadias patients	5.71 ± 3.92
√ Controls	
Hypospadias type (n = 40)	1 (2.5)
√ Distal	5 (12.5)
♣ Glandular	14 (35)
♣ Subcoronal	13 (32.5)
♣ Midshaft	6 (15)
√ Proximal	1 (2.5)
♣ Penoscrotal	
♣ Scrotal	
♣ Perineal	
Penile curvature (n = 40)	14 (35)
√ Mild (< 30 degree)	12 (30)
√ Moderate (30–60 degree)	14 (35)
√ Severe (> 60 degree)	
Penoscrotal transposition	2 (5)
√ Yes	38 (95)
√ No	
Bifid scrotum	6 (15)
√ Yes	34 (85)
√ No	

Table 2
Comparison of COL1A1 and COL6A1 expressions base on hypospadias type and penile curvature severity.

Hypospadias type				
Gene	Distal Hypospadias	Proximal Hypospadias	Control	p-value
COL1A1	0.88 (0.38–2.53)	0.76 (0.33–2.57)	1.85 (0.24–4.61)	0.024*
COL6A1	0.54 (0.16–4.35)	0.57 (0.18–1.38)	0.93 (0.17–4.06)	0.018*
Penile curvature severity				
Gene	Mild (n = 14)	Moderate (n = 12)	Severe (n = 14)	p-value
COL1A1	1.56 (0.47–2.57)	0.69 (0.33–1.59)	0.73 (0.38–1.11)	0.003*
COL6A1	0.90 (0.16–4.35)	0.57 (0.23–1.04)	0.44 (0.18–0.89)	0.037*
*, $p < 0.05$ (Kruskal Wallis test)				

Moreover, there was no significant difference of COL1A1 and COL6A1 expressions either between proximal and distal hypospadias or severe and moderate penile curvature (Table 3). Furthermore, there was a moderate correlation between *COL1A1* and *COL6A1* expressions ($r = 0.458$, $p = 0.000$).

Table 3
Comparison of COL1A1 and COL6A1 expressions between hypospadias groups, moderate and severe penile curvature.

Gene	Hypospadias type		p	95% CI		Penile Curvature		p*	95% CI	
				Lower	Upper				Lower	Upper
COL1A1	Distal	Proximal	0.957	0.963	0.972	Moderate	Severe	0.877	0.896	0.911
COL6A1	Distal	Proximal	0.570	0.570	0.596	Moderate	Severe	0.123	0.125	0.142

Gen	Control	Hypospadia Type		Penile curvature		
		Distal Hypospadia	Proximal Hypospadia	Mild	Moderate	Severe
GAPDH						
COL1A1						
COL6A1						

*Mann-Whitney test

Discussion

Here, we are able to show the downregulated *COL1A1* and *COL6A1* expressions in hypospadias, moderate and severe penile curvature patients. These results are consistent with the previous research which found that the mean number of total collagen fibers of dartos tissue in hypospadias was lower but has thicker fibers compared to normal patients. The study also found the abnormality of hypospadias tissue which gave the thick and inelastic character although it was not followed by the increasing of collagen number (11). Another study showed no evidence of fibrous band or dysplastic tissue in subepithelial biopsies analyzed in 17 prepubertal boys undergoing hypospadias repair (12). Eros *et al.* proposed no difference in collagen intensity between normal areas and under urethral plate of hypospadias patients (13).

Type 1 Collagen is the most abundant collagen. It is the predominant component of interstitial membranes. It is also associated with fibrosis and fibrogenesis (14). Hayashi et al., (2011) explained the excess deposition of collagen type 1 occurs in the maturation phase of scar formation, making the fibrils

become large and stiff bands. It may cause less tumescence in patients with scar formation or fibrosis of the corpus cavernosum penis (6).

This paper also investigated the gene expression of *COL6A1* as the most studied type of collagen in the beaded- filament-forming collagens group. It plays a central role in cell attachments and connection between tissues and surrounding matrix (15). Type 6 collagen also regulates fibrogenesis by modulating the interactions among cells. It stimulates the activation of mesenchymal cells into myofibroblasts resulting the extracellular matrix deposition and tissue fibrosis (15). In lung fibrosis, type 6 collagen is expressed increasingly (15). However, this study found decreasing gene expression of COL6A1 in hypospadias groups compared to the control group. It might relate with the downregulation of COL1A1which was found in this paper, suggesting type 6 collagen is bound together to the sides of type 1 collagen to form thicker collagen fibers (7). We found significant positive correlation between COL1A1 and COL6A1.

The etiology of chordee in hypospadias remains unclear. Resection of dartos tissue usually can straighten the penis in patients with chordee and buried penis, suggesting the pathophysiology of these anomalies is related to dartos tissue (4). The composition of dartos fibromuscular tissue determines tissue elasticity and the skin mobility. (4, 12)

Collagen is a part of tissue backbone. During tissue turnover, it is formed and degraded to maintain tissue health and homeostasis. Imbalance of that process lead to fibrosis. Fibrosis is known as excessive formation of connective tissue which damage the structure and function of its tissue (16). Chordee occurs because of fibrosis in dartos tissue which disrupts the tissue elasticity and results in the curving of penile (17).

We also investigated the statistical difference of *COL1A1* and *COL6A1* expressions among groups based on penile curvature in hypospadias group. Mostly, severe forms of hypospadias are related to a significant chordee and a urethral meatus located proximal to the midshaft of the penis (18). Another study proposed that severity of chordee is generally proportional to the degree of hypospadias (19). Many factors, such as abnormal development of the urethral plate, fibrotic mesenchymal tissue at the urethral meatus, and ventral-dorsal corporal disproportion may be interconnected resulting different degree on final severity of curvature in each patient (20).

Conclusion

Aberrant *COL1A1* and *COL6A1* expressions might affect the abnormality of dartos tissue and penile curvature severity in hypospadias patients.

Abbreviations

ECM : Extracellular matrix

COL1A1 : Collagen 1 subtype alpha 1

COL6A1 : Collagen 6 subtype alpha 1

GAPDH : Glyceraldehyde 3-phosphate dehydrogenase

qPCR : reverse-transcriptase polymerase chain reaction

Declarations

Ethics approval and consent to participate

The Ethical Committee of Faculty of Medicine, Universitas Gadjah Mada/Dr. Sardjito Hospital gave approval for this study (KE/FK/0699/EC). Written informed consent was obtained from all parents for participating this study.

Consent to publish

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in the submission. The raw data can be requested to the corresponding author.

Competing interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article

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Authors' Contribution

PY collected the patient data and PY, IS, NAD analyzed and interpreted data. PY, WA, NA RP, FP performed PCR examination of the dartos tissue. PY, IS, NAD was a major contributor in writing the manuscript. All authors' read and agreed for the final manuscript.

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Figures

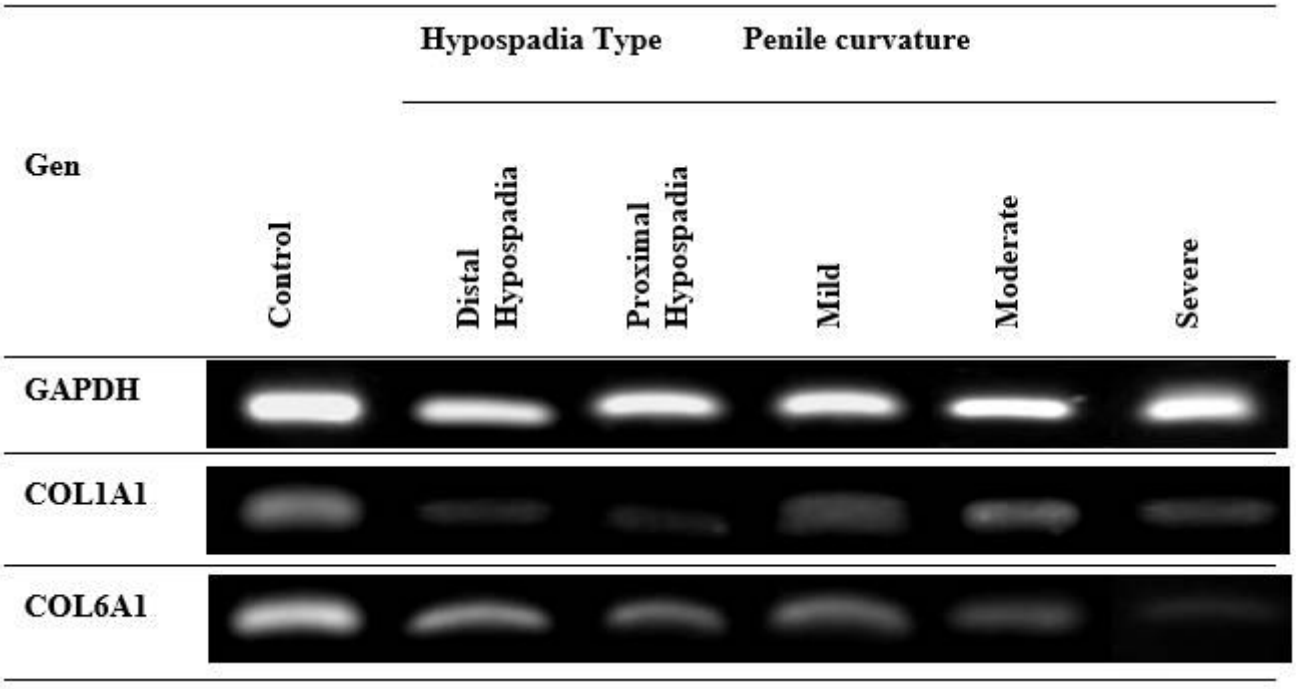

Figure 1

Representative of qPCR products of COL1A1 and COL6A1. GAPDH was used as reference gene.