

ORIGINAL PAPER

Correlation between histopathology properties of dartos tissue and the severity of penile curvature in hypospadias

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Summary *Purpose: Hypospadias, one of the congenital anomalies commonly associated with some degrees of ventral penile curvature that may arise from malformation of dartos fascia, the chordee. Our study aims to determine the correlation between the histopathology properties of dartos fascia and the severity of ventral penile curvature in hypospadias.*

Materials and methods: One hundred hypospadias patients with various degrees of ventral penile curvature were included in this cross-sectional analytical study from 2020 to 2022. During hypospadias repair, ventral dartos fascia was excised and analyzed for the degree of collagen thickness and the severity of the fibrotic condition.

Results: Out of 100 patients, the mean age was 6.58 ± 4.28 years, who were classified as mild to moderate (66%) and severe (34%) ventral curvature cases. The analyses showed significant differences in the severity of fibrotic condition and collagen thickness of dartos fascia to the severity of penile ventral curvature with p-values of 0.002 and 0.017, respectively. Conclusions: The difference in histopathology properties of dartos fascia may affect the severity of penile curvature in hypospadias patients.

KEY WORDS: Hypospadias; Chordee; Penile curvature; Collagen; Fibrotic.

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INTRODUCTION

Hypospadias is a congenital anomaly found in children characterized by an abnormal location of urethral opening on the ventral side of the penis. The incidence of hypospadias varies around the world, with a prevalence of about 1 case in every 250 male newborn babies. This malformation is caused by abnormal development of the ventral side of the penis, hence an abnormal proximal position of the urethral opening (1).

The abnormality has been classified into several categories. Based on the position of the urethral opening, hypospadias can be differentiated into proximal and distal types. Ventral curvature, the chordee, is another asso-

ciated condition found in hypospadias, mostly the proximal type, which is the proximal part of urethral folds that were fused incompletely and left an opening in the ventral penis in males (2, 3). Chordee may arise from the inadequacy or disorder of the dartos fascia, on the ventral side of the penis. The severity of the chordee may be associated with the degree of dartos fascia abnormality (4). In hypospadias patients, the composition of the extracellular matrix (ECM) was varied and different compared to the non-hypospadias patients, in which the main components are collagens, elastic fibers, and proteoglycans/glycosaminoglycans (GAGs). These composition changes might contribute to the thickening and stiffness of the tissue. A previous study reported that the dartos fascia in hypospadias had thicker collagen fibers than a normal penis (5).

To the best of our knowledge, there is no study reporting if there is any correlation between the degree of collagen thickness and the severity of the fibrotic condition of dartos fascia to the severity of ventral curvature. Therefore, this study aimed to compare the degree of collagen thickness and the severity of the fibrotic condition to the severity of ventral curvature in hypospadias.

MATERIALS AND METHODS

A cross-sectional analytical study was performed on 100 hypospadias patients with various degrees of chordee who underwent urethroplasty between 2020 and 2022. Patients were differentiated into two groups based on the severity of penile curvature degree, namely mild to moderate (< 60 degrees) and severe (> 60 degrees). The penile curvature degree was measured, twice at least, using a goniometer intraoperatively before performing urethroplasty and a single surgeon (GWKD) performed urethroplasty for this study. Excision of ventral dartos fascia, including tunica albuginea, which manifests as chordee, was performed during surgery with a U-shape incision, which was a wide excision of urethra of about 8 mm in length, which started at 2 mm from proximal tip of urethral meatus to 2 mm from distal urethral meatus. These tissues were sent to the pathology laboratory to assess the fibrotic and collagen density conditions. Patients who underwent re-urethro-

plasty due to a failed previous operation were excluded.

The study has been approved by the research ethics committee of the Faculty of Medicine, Universitas Udayana, Prof. I.G.N.G. Ngoerah General Hospital, Denpasar with No. 2773/UN14.2.2VII.14/LT/2022, and written informed consent has been acquired from the parents.

The staining and evaluation of dartos specimens were conducted by a single pathologist (NWW) without prior information regarding the clinical condition of patients. The Masson's trichrome (MST) and hematoxylin and eosin (H&E) staining were performed to assess the collagen fibers thickness and the severity of the fibrosis, respectively. The images were then enlarged 200 times and five random points were marked to evaluate the specimen thoroughly. Regarding the thickness of collagen, patients were divided into two groups: thin to moderate and thick. Moreover, patients were also assigned into two groups in terms of the severity of fibrotic conditions, namely mild to moderate and severe. The cut-off points of thin to moderate and thick/severe are below or above 60% per high power field (HPF) for both variables. These percentages were obtained by dividing the area occupied by collagen by the area of the entire high power field.

The Chi-square and the alternative Fisher's exact tests were used to analyze the relationship between the severity of chordee and collagen thickness and fibrotic severity on dartos tissue. P-value < 0.05 was considered statistically significant.

RESULTS

The characteristics of all patients in our institution are shown in Table 1. The mean age of 100 patients was 6.58 + 4.28 years and they were classified into mild to moderate (66%) and severe (34%) ventral curvature cases. Moreover, in terms of collagen thickness of dartos fascia, there were 76% and 24% cases with thin to moderate and

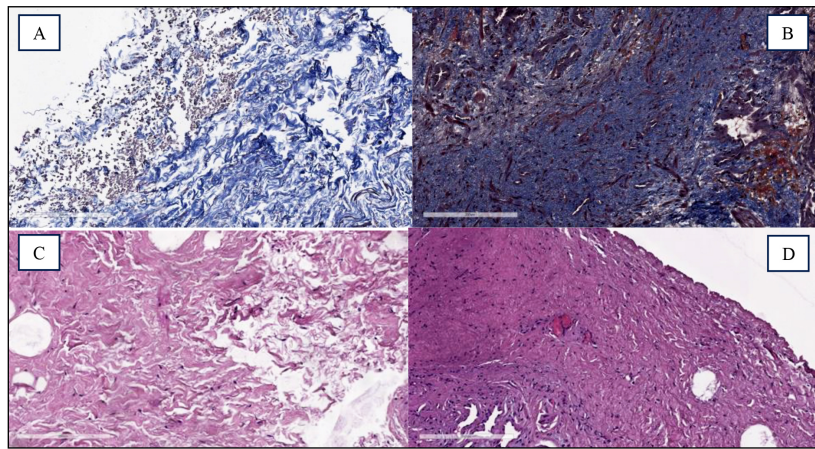


Figure 1.

The MST staining of collagen fibers (200x), the green arrow points to the thin to moderate collagen fibers in Figure (A) and thick collagen fibers in Figure (B). The H&E staining of fibrotic changes (200x), the green arrow points to the mild to moderate fibrosis in Figure (C) and severe fibrosis in Figure (D).

thick collagen density, respectively. In addition, 87% and 13% of patients suffered from mild to moderate and severe fibrotic conditions of their dartos fascia, respectively. Representation of thin to moderate and thick collagen density as well as mild to moderate and severe fibrosis, shown in Figure 1.

The analyses showed significant differences in the severity of fibrotic and collagen thickness of dartos fascia to the severity of ventral curvature with p-values of 0.002 and 0.017 respectively, shown in Table 2.

Table 2.

Analysis of collagen thickness and fibrotic severity on ventral curvature.

Variable	Ventral curvature degree		P-value
	Mild-moderate	Severe	
Fibrotic severity of dartos fascia			
Mild-moderate	61	26	0.002 *
Severe	5	8	
Collagen thickness of dartos fascia			
Thin-moderate	55	21	0.017 **
Thick	11	13	

*: significant result (Fisher's exact test); **: significant result (Chi-square test).

Table 1.

Characteristics of all patients.

Variable	
Hypospadias patients (n = 100)	
Mean age (year + SD)	6.58 + 4.28
Ventral curvature, n (%)	
Mild-moderate	66 (66)
Severe	34 (34)
Fibrotic severity of dartos fascia, n (%)	
Mild-moderate	87 (87)
Severe	13 (13)
Collagen thickness of dartos fascia, n (%)	
Thin-moderate	76 (76)
Thick	24 (24)

DISCUSSION

A previous study revealed that the number collagen fibers of dartos fascia was significantly lower but the fibers were thicker in hypospadias compared to normal penis. Moreover, the reticulin to total collagen ratio was higher in hypospadias compared to normal penis. Reticulin, a type III collagen fibers, was found to increase during early stage of life and early healing condition (6). At the molecular level, Yuri *et al.* showed the downregulation of several types of collagen gene expressions compared to normal patients. COL1A1 and COL6A1 were also significantly downregulated in the moderate and severe chordee

groups compared to the mild chordee groups, with p-values of 0.003 and 0.037, respectively (7, 8). Therefore, we assessed the thickness of collagen fibers in hypospadias in several degrees of ventral curvature. Our study reported that collagen fibers of dartos fascia in hypospadias were significantly thicker in severe compared to mild to moderate ventral curvature. As far as we know, this is the first study comparing the histopathology aspect of dartos fascia in various degrees of ventral curvature of hypospadias. Fibrotic tissue leads to thickening and scarring of the tissue it affects (9, 10). Fibrotic tissue is a collection of *extracellular matrix* (ECM) and it may appear in various degree of elasticity and thickness that depends on its properties of collagen and elastin (11). Fibrosis is known as a disproportionate development of connective tissue that disrupts the structure and function of any tissue (12). Chordee exists due to the fibrosis of dartos fascia that results in elasticity disruption and leads to penile bending (13). Moreover, we reported the distinction of dartos fascia properties in various degrees of ventral curvature of hypospadias patients, especially in collagen thickness and fibrosis severity. The more severe ventral curvature degree patients suffered, the more severe and thicker the fibrotic condition and collagen thickness they would have. Hypospadias patients with severe ventral curvature had more abnormal and inelastic dartos fascia tissue that led to more severe ventral bending of the penile.

Histopathology study on the hypospadias urethral plate revealed the characteristic of well-built connective tissue with adequate vascularization and no signs of fibrosis on the urethral plate (14). Moreover, there was no difference in collagen density in patients with hypospadias compared to the normal patient (15). However, the readouts of our study are different from those of previous studies. Previous studies evaluated the histopathology of the urethral plate, on the other hand, our study assessed the histopathology of the dartos fascia especially taken from the ventral side of the penile.

In the current literature, there are no guidelines about the definition of collagen fiber thickness and fibrotic changes in micrometers, which might be a potential bias due to tissue sampling and handling for microscope analysis differences in size.

Several pieces of literature have reported the abnormality of dartos fascia in hypospadias, buried penis and also in epispadias cases (3, 16). *Atmoko et al.* showed a reduction of total collagen to elastin and also an increased ratio of reticulin fibers in dartos fascia of hypospadias and buried penis (3). Moreover, there were thicker collagen and abnormal smooth muscle action distribution in hypospadias and buried penis compared to normal penile (3, 16). To the best of our knowledge, this is the first study to compare the histopathology of dartos fascia based on the severity of ventral curvature in hypospadias patients. This study showed that severe ventral curvature is correlated with thicker collagen fibers and more severe fibrosis condition of dartos fascia compared with mild to moderate ventral curvature in hypospadias patients. A more progressive excision of the chordee is warranted in severe ventral curvature penile during hypospadias repair. In fact, total excision of the chordee is mandatory during urethroplasty regardless of the degree of penile curvature

as this is an abnormal tissue. Leaving even a small remnant of chordee during reconstructive surgery may increase the risk of complications following surgery (17). We realized that there was a more objective measurement of fibrosis based upon immunohistochemical staining and/or quantification of relative protein expression such as collagen types and elastin. Therefore, we recommended that further study is needed to evaluate the composition of collagen in dartos fascia based on the severity of chordee using an electron microscope with specific immunohistochemical staining. Since we did not focus on and discuss the surgical outcomes in our study, we also recommended further study to assess the complication post-surgical to evaluate the outcome, which is not only studies at the protein level and molecular studies

CONCLUSIONS

There is a difference in the properties of dartos fascia in severe ventral curvature compared with mild to moderate ventral curvature of hypospadias patients, especially in the thickness of collagen fibers and severity of the fibrotic condition. The difference in histopathology properties of dartos fascia may affect the severity of penile curvature in hypospadias patients.

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