COMPREHENSIVE HISTOPATHOLOGICAL PROPERTIES OF DARTOS TISSUE ASSOCIATED WITH HYPOSPADIAS SEVERITY AND CHORDEE: A PROSPECTIVE STUDY

Putu Angga Risky Raharja[1,2,*], Ponco Birowo[2], Lisnawati Rachmadi[3], Heri Wibowo[4], Aria Kekalih[5], Gede Wirya Kusuma Duarsa[6], Irfan Wahyudi[2]

[1]Doctoral program in Medical Sciences, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia, [2]Department of Urology, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jakarta, Indonesia, [3]Department of Anatomical Pathology, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jakarta, Indonesia, [4]Department of Parasitology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia, [5]Department of Community Medicine, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia, [6]Department of Urology, Faculty of Medicine, Universitas Udayana, Prof. Dr. I.G.N.G Ngoerah Hospital, Bali, Indonesia

eISSN: 2828-4070
Proceedings ICE on IMERI. 2024.
Received: December 7th, 2024
Accepted: December 18th, 2024
Published online: January 17th , 2025

Corresponding Author
Name : Putu Angga Risky Raharja
Email : anggariskyraharja@gmail.com

Abstract

Background: The cause of chordee in hypospadias is not fully understood. Dartos fascia resection has been shown to correct penile curvature, suggesting its potential involvement. This study aimed to evaluate the histopathological properties of dartos tissue and their association with hypospadias severity and chordee. **Methods and Materials:** Dartos tissue samples were collected from 37 children with hypospadias and ten controls undergoing circumcision between March and October 2024. Histopathological analysis using hematoxylin-eosin and Masson's trichrome staining assessed for collagen, smooth muscle, and vascular density. Assessment and quantification of all histopathological components were blinded. **Results:** The hypospadias group showed lower collagen density (60.85±6.75% vs. 67.31±4.61%; p=0.003) and higher smooth muscle density (7.34±4.92% vs. 2.51±2.24%; p<0.001) compared to controls, with no significant difference in vessel density. Sub-analysis showed severe chordee was linked to lower collagen density compared to mild (Mean Difference [MD] -6.24±2.41%; p=0.014) and moderate chordee (MD -5.73±2.54%; p=0.031). **Conclusions:** The dartos tissue in hypospadias patients exhibited lower collagen density and higher smooth muscle density, with chordee severity specifically linked to decreased collagen density.

Keywords
Chordee, collagen density, dartos tissue, histopathological, hypospadias.

Introduction

Hypospadias ranks among the most frequent congenital anomalies of the male urogenital system, affecting approximately 1 in 150 to 300 live births.[1,2] This condition is defined by the abnormal positioning of the urethral opening on the ventral surface of the penis, often accompanied by a ventral penile curvature, known as chordee, and a distinctive dorsal "hooded" foreskin with a deficiency on the ventral side.[3] Among the triad of features defining hypospadias, chordee is a key component and can be classified into superficial and deep types.[4,5] Superficial chordee is associated with abnormalities in the superficial fascia. In contrast, deep chordee involves structural disruptions in the deep fascia, urethral plate, corpus spongiosum, or tunica albuginea and may result from disproportionate development of the corpora.[5]

The mechanisms underlying chordee in hypospadias remain poorly understood; however, the correction of penile curvature through dartos fascia resection suggests its involvement.[6] Studies have noted that the dartos fascia in hypospadias is often thicker and less elastic, yet the relationship between its histopathological features and chordee severity has not been extensively studied.[7,8] Currently available studies are qualitative, with limited quantitative analyses.[9] Furthermore, the potential benefits of dartos fascia resection during hypospadias repair have been suggested but not fully explored.[10] This study aims to address these gaps by examining the histopathological characteristics of the dartos tissue and their association with hypospadias severity and the degree of chordee.

Materials and Methods

Study Design

This prospective study compared the dartos tissue of patients with hypospadias to the dartos tissue of normal patients undergoing circumcision. The research was carried out at Cipto Mangunkusumo Hospital in Jakarta, Indonesia, from March to October 2024, following approval by the Medical Research Ethics Committee of Universitas Indonesia.

Participants

This study involved 37 children with a confirmed diagnosis of hypospadias and ten boys serving as controls, all of whom underwent elective circumcision. Inclusion criteria for the hypospadias group included a definitive diagnosis, suitability for surgical repair, and the absence of any previous surgical interventions. Control participants were children undergoing circumcision for non-pathological reasons, with no evidence of penile anomalies. Patients with a history of previous genital surgeries or those with inadequate tissue samples for analysis were excluded from the study. Written informed consent was obtained from the parents or legal guardians of all participants before study enrollment.

Data Collection

Dartos tissue samples were obtained from the ventral side near the meatal region in both study groups. In the hypospadias group, tissue samples were collected during

penile degloving, whereas in the control group, samples were harvested during the circular incision of the prepuce. For the hypospadias group, chordee measurements were performed using a goniometer during an artificial erection test and classified according to the GMS hypospadias scoring system into mild (<30°), moderate (30°–60°), and severe (>60°) categories.[11]

All dartos tissue specimens were fixed in 10% buffered formalin and subsequently embedded in paraffin for histological examination. Hematoxylin and eosin (H&E) staining and Masson's trichrome staining were employed to assess the collagen, smooth muscle, and vascular density within the dartos tissue. Whole-slide images were digitized and analyzed using the built-in pixel classifier in QuPath software. All histopathological assessments and quantifications were conducted in a blinded manner.

Statistical Analysis

The data were processed and analyzed using the Statistical Package for the Social Sciences (SPSS) software, version 29.0 (IBM Corporation). Descriptive statistics were used to summarize the data, with frequencies reported for categorical variables and means or medians presented for continuous variables based on the normality of the data distribution. Comparative analysis of collagen content, smooth muscle, and vascular density in the dartos tissue was conducted using either the Independent T-test or the Mann-Whitney U-test. Subgroup analyses categorized by chordee severity (mild, moderate, or severe) were conducted using either a One-Way ANOVA or a Kruskal-Wallis test, as appropriate. When significant differences were identified, appropriate post-hoc analyses were conducted. Statistical significance is considered if $p<0.05$.

Results

A total of 47 dartos tissue samples were included in the analysis, of which 37 were obtained from patients with hypospadias and 10 from control participants undergoing circumcision. Table 1 demonstrates that the two groups were similar in age and body mass index (BMI). In the hypospadias group, proximal cases were more common than distal, and the severity of chordee was distributed relatively evenly across mild, moderate, and severe categories. Furthermore, 27% of the hypospadias patients had undergone hormonal therapy prior to surgery.

Table 1. Baseline Characteristics of Study Participants

	Hypospadias group (N = 37)	Control group (N = 10)	P value
Age (years)	4.64 ± 2.47	4.00 ± 2.10	0.453
Body mass index (kg/m^2)	16.63 (9.01 – 28.78)	16.43 (14.84 – 26.88)	0.654
Hypospadias classification:			
Distal	15 (40.5%)	N/A	N/A
Proximal	22 (59.5%)		
Chordee classification:			
Mild	12 (32.4%)	N/A	N/A
Moderate	10 (27.0%)		
Severe	15 (40.6%)		

Hormonal therapy:			
Yes	10 (27.0%)	N/A	N/A
No	27 (73.0%)		

Histological analysis revealed significant differences in dartos tissue composition between the hypospadias and control groups, as illustrated in Figure 1. Quantitative assessment showed that collagen density was significantly lower in the hypospadias group compared to the controls (60.85±6.75% vs. 67.31±4.61%; p=0.003). Smooth muscle density was markedly elevated in the hypospadias group (7.34±4.92% vs. 2.51±2.24%; p<0.001), whereas vessel density did not differ significantly between the groups (Table 2). Further subgroup analysis based on chordee severity demonstrated that severe chordee was associated with significantly lower collagen density compared to mild (mean difference [MD] -6.24±2.41%; p=0.014) and moderate chordee (MD -5.73±2.54%; p=0.031) (Table 3).

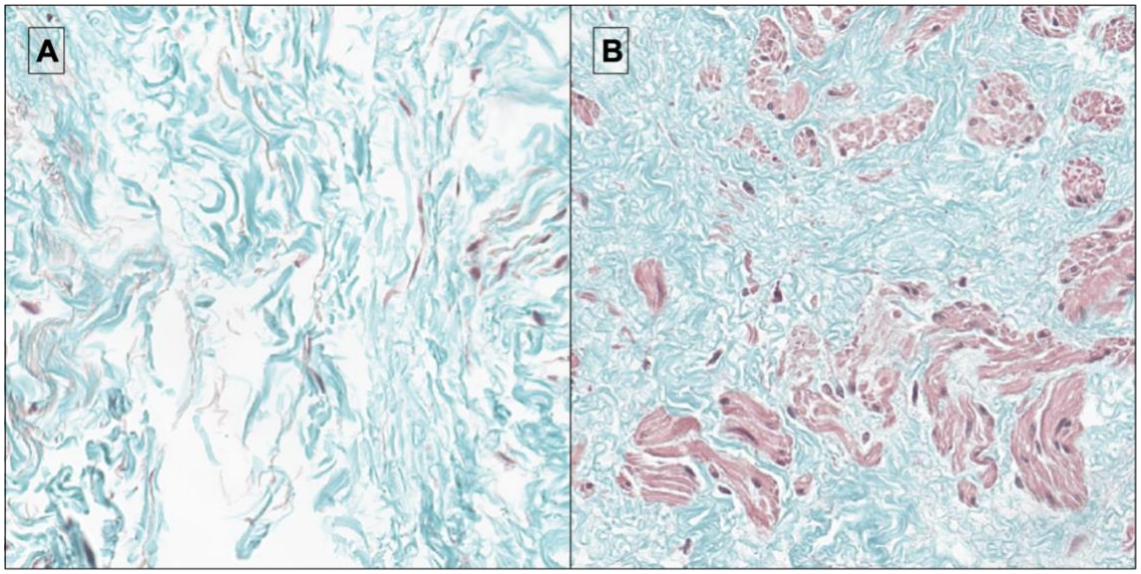

Figure 1. Histological Differences in Dartos Tissue Between Hypospadias (A) and Control Groups (B) stained with Masson's Trichrome's (100x magnification)

Table 2. Quantitative Analysis of Dartos Tissue Composition

	Hypospadias group (N = 37)	Control group (N = 10)	P value
Collagen density (%)	60.85 ± 6.75	67.31 ± 4.61	**0.003**
Smooth muscle density (%)	7.34 ± 4.92	2.51 ± 2.24	**<0.001**
Vessel density (%)	5.26 ± 2.77	4.81 ± 2.85	0.654

Table 3. Quantitative Subgroup Analysis of Dartos Tissue Composition Across Chordee Severity

	Mild Chordee (N = 12)	Moderate Chordee (N = 10)	Severe Chordee (N = 15)	P value
Collagen density (%)	63.52 ± 4.44	63.01 ± 6.78	57.28 ± 7.01	**0.024**
Smooth muscle density (%)	6.26 ± 4.88	6.64 ± 4.23	8.68 ± 5.38	0.402
Vessel density (%)	6.10 ± 2.29	5.98 ± 2.47	4.09 ± 3.04	0.106

Discussion

This study provides a detailed analysis of the histopathological characteristics of dartos tissue in patients with hypospadias compared to controls. Our findings revealed that dartos tissue in hypospadias patients is characterized by significantly lower collagen density, higher smooth muscle density, and abnormal muscle fiber architecture. Additionally, we observed that the severity of the chordee is closely associated with decreased collagen density, highlighting its potential role in determining penile curvature.

Our observation of reduced collagen density in the hypospadias group aligns with previous studies.[7,10] Atmoko *et al.* observed that while the average number of collagen fibers in the dartos fascia was reduced in patients with buried penis and hypospadias compared to normal controls, the fibers themselves appeared thicker.[10] Similarly, Yuri *et al.* demonstrated downregulated COL1A1 and COL6A1 expressions in hypospadias patients.[7] Collagen, essential for tissue structure, undergoes constant turnover to maintain homeostasis.[12,13] Disruption of this process leads to fibrosis, marked by excessive connective tissue deposition that impairs function.[14] In hypospadias, dartos tissue fibrosis reduces elasticity, causing chordee and penile curvature.

Our subgroup analysis revealed that severe chordee is associated with reduced collagen density. Similarly, Yuri *et al.* reported significant downregulation of COL1A1 and COL6A1 in moderate and severe chordee groups.[7] Severe hypospadias is typically marked by significant chordee and the urethral opening located closer to the base of the penis.[15] Generally, the severity of chordee correlates with the degree of hypospadias.[16] The reduced collagen density observed in severe chordee may be linked to increased fibrosis, which could contribute to the pronounced curvature.

The composition of dartos fibromuscular tissue plays a key role in determining tissue elasticity and skin mobility.[6,17] Our analysis revealed increased muscle density in hypospadias and abnormal muscle fiber. This observation aligns with the findings of Spinoit *et al.*, highlighting structural histological abnormalities underlying penile curvature beyond the mere presence or absence of fibrosis.[6]

Our analysis revealed no significant difference in blood vessel density between the hypospadias and control groups, which is consistent with previous studies.[10,18] Yuri *et al.* similarly reported comparable VEGF expression levels in patients with hypospadias and the control group.[18] Despite the abnormal development of specific extracellular matrix structures in dartos tissue, this study demonstrated that the tissue maintains good vascularization.

This study has several limitations. First, the relatively small sample size, particularly in the control group, may limit the generalizability of our findings. Second, the analysis was focused primarily on histological characteristics without incorporating advanced imaging or molecular techniques that could provide a more comprehensive understanding of dartos tissue abnormalities. Third, the study did not assess functional outcomes, such as surgical or clinical results, which could offer additional insights into the implications of

these histopathological findings. Future studies incorporating larger cohorts, diverse populations, and functional evaluations are needed to strengthen the clinical applicability of our results.

Conclusions

The dartos tissue in hypospadias patients exhibited lower collagen density and higher smooth muscle density, with chordee severity specifically linked to decreased collagen density. These findings suggest that structural abnormalities in dartos tissue contribute to the pathogenesis of chordee and highlight the importance of considering tissue properties in the surgical management of hypospadias.

Conflict of Interests

The authors declare no conflicts of interest regarding the publication of this manuscript.

Acknowledgments

This article was presented at the 9th International Conference and Exhibition on Indonesian Medical Education and Research Institute (9th ICE on IMERI) 2024, Faculty of Medicine, Universitas Indonesia. We are grateful for the outstanding assistance provided by the committee of the 9th ICE on IMERI 2024 throughout the preparation of the manuscript and the peer-review process.

References

1. Elliott CS, Halpern MS, Paik J, Maldonado Y, Shortliffe LD. Epidemiologic trends in penile anomalies and hypospadias in the state of California, 1985-2006. J Pediatr Urol 2011;7:294–8. https://doi.org/10.1016/j.jpurol.2011.03.006.
2. Springer A, van den Heijkant M, Baumann S. Worldwide prevalence of hypospadias. J Pediatr Urol 2016;12:152.e1-7. https://doi.org/10.1016/j.jpurol.2015.12.002.
3. Halaseh SA, Halaseh S, Ashour M. Hypospadias: A Comprehensive Review Including Its Embryology, Etiology and Surgical Techniques. Cureus 2022;14:e27544. https://doi.org/10.7759/cureus.27544.
4. Nordenvall AS, Frisén L, Nordenström A, Lichtenstein P, Nordenskjöld A. Population based nationwide study of hypospadias in Sweden, 1973 to 2009: incidence and risk factors. J Urol 2014;191:783–9. https://doi.org/10.1016/j.juro.2013.09.058.
5. Hadidi AT. The Urethral Plate and Chordee BT - Hypospadias Surgery: An Illustrated Textbook. In: Hadidi AT, editor., Cham: Springer International Publishing; 2022, p. 185–235. https://doi.org/10.1007/978-3-030-94248-9_8.
6. Spinoit A-F, Van Praet C, Groen L-A, Van Laecke E, Praet M, Hoebeke P. Congenital penile pathology is associated with abnormal development of the dartos muscle: a

prospective study of primary penile surgery at a tertiary referral center. J Urol 2015;193:1620–4. https://doi.org/10.1016/j.juro.2014.10.090.

7. Yuri P, Gunadi, Lestari RP, Fardilla FP, Setyaningsih WAW, Arfian N, et al. The impact of COL1A1 and COL6A1 expression on hypospadias and penile curvature severity. BMC Urol 2020;20:189. https://doi.org/10.1186/s12894-020-00760-w.
8. Makovey I, Higuchi TT, Montague DK, Angermeier KW, Wood HM. Congenital Penile Curvature: Update and Management. Curr Urol Rep 2012;13:290–7. https://doi.org/10.1007/s11934-012-0257-x.
9. Van der Putte SCJ. Hypospadias and associated penile anomalies: a histopathological study and a reconstruction of the pathogenesis. Journal of Plastic, Reconstructive and Aesthetic Surgery 2007;60:48–60. https://doi.org/10.1016/j.bjps.2006.05.020.
10. Atmoko W, Shalmont G, Situmorang GR, Wahyudi I, Tanurahardja B, Rodjani A. Abnormal dartos fascia in buried penis and hypospadias: Evidence from histopathology. J Pediatr Urol 2018;14:536.e1-536.e7. https://doi.org/10.1016/j.jpurol.2018.04.034.
11. Merriman LS, Arlen AM, Broecker BH, Smith EA, Kirsch AJ, Elmore JM. The GMS hypospadias score: Assessment of inter-observer reliability and correlation with post-operative complications. J Pediatr Urol 2013;9:707–12. https://doi.org/10.1016/j.jpurol.2013.04.006.
12. Karsdal M. Biochemistry of collagens, laminins and elastin: structure, function and biomarkers. Elsevier; 2023.
13. Henriksen K, Karsdal MA. Type I collagen. Biochemistry of collagens, laminins and elastin, Elsevier; 2024, p. 1–11.
14. Montag S, Palmer LS. Abnormalities of penile curvature: chordee and penile torsion. The Scientific World Journal 2011;11:1470–8.
15. Abbas TO, Mahdi E, Hasan A, AlAnsari A, Pennisi CP. Current status of tissue engineering in the management of severe hypospadias. Front Pediatr 2018;5:283.
16. Azmy AF. Chordee (penile curvature). Hypospadias Surgery: An Illustrated Guide, Springer; 2004, p. 115–8.
17. Snodgrass W, Patterson K, Plaire JC, Grady R, Mitchell ME. Histology of the urethral plate: Implications for hypospadias repair. Journal of Urology 2000;164:988–90. https://doi.org/10.1016/s0022-5347(05)67233-3.
18. Yuri P, Gunadi, Lestari RP, Fardilla FP, Dachlan I. Expression of mRNA vascular endothelial growth factor in hypospadias patients. BMC Urol 2021;21:163. https://doi.org/10.1186/s12894-021-00930-4.