

Comparative Analysis of MMP-1 and α -SMA Expression in Ventral and Dorsal Dartos Tissue of Patients With Hypospadias and Chordee

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Abstract

Background

Hypospadias-associated chordee is believed to result from abnormal extracellular matrix remodeling and connective tissue organization within the penile dartos fascia. However, molecular differences between ventral and dorsal dartos compartments remain poorly understood. This study aimed to compare the gene expression of matrix metalloproteinase-1 (MMP-1) and alpha-smooth muscle actin (α -SMA) between ventral and dorsal dartos tissues in patients with hypospadias and chordee.

Methods

A cross-sectional observational study was conducted over a 24-month period at a tertiary referral center in Indonesia. Thirty-three pediatric patients with hypospadias and chordee undergoing urethroplasty were enrolled. Ventral and dorsal dartos tissue specimens were collected intraoperatively. Relative mRNA expression of MMP-1 and α -SMA was quantified using quantitative real-time polymerase chain reaction (qRT-PCR), normalized to β -actin, and analyzed using the $2^{-\Delta\Delta C_t}$ method. Comparisons between tissue compartments were performed using appropriate statistical tests, with statistical significance defined as $p < 0.05$.

Results

Ventral dartos tissue demonstrated significantly lower MMP-1 expression than dorsal tissue (median fold change: 0.565 vs. 0.609; $p = 0.035$). Conversely, α -SMA expression was significantly higher in ventral tissue than in dorsal tissue (median fold change: 1.242 vs. 0.574; $p < 0.001$). These findings indicate distinct molecular profiles between ventral and dorsal dartos tissues in patients with hypospadias and chordee.

Conclusions

Significant regional differences in MMP-1 and α -SMA gene expression were identified between ventral and dorsal dartos tissues. Reduced MMP-1 expression together with increased α -SMA expression in ventral tissue suggests altered extracellular matrix remodeling and enhanced fibrotic activity that may contribute to the pathogenesis of penile curvature. These findings provide preliminary molecular evidence supporting compartment-specific biological differences within dartos tissue and warrant further investigation using protein-level analyses and functional studies.

Introduction

Hypospadias is one of the most common congenital anomalies of the male genitalia, affecting approximately 1 in every 200–300 live male births worldwide ^(1,2). The condition is characterized by an ectopic ventral urethral meatus and is frequently associated with ventral penile curvature (chordee), abnormal preputial development, and varying degrees of structural abnormalities of the penis. Although nearly 70% of cases involve distal hypospadias, proximal forms are more commonly associated with severe chordee and complex anatomical defects that often require extensive reconstructive surgery ^(1,3,7).

The etiology of hypospadias is multifactorial and involves complex interactions among genetic susceptibility, endocrine regulation, and environmental exposures during fetal development ^(2,5,6,7). Increasing evidence suggests that disruption of androgen signaling and exposure to endocrine-disrupting chemicals may interfere with normal genital tubercle development, leading to abnormal urethral formation ^(2,5,6,7). In addition to these developmental mechanisms, alterations in connective tissue composition and extracellular matrix (ECM) remodeling have emerged as important contributors to the pathogenesis of hypospadias-associated chordee ^(8,9,10,11,12).

The penile dartos fascia is a specialized fibrovascular connective tissue layer that provides elasticity and mobility to the penile shaft and is routinely manipulated during hypospadias repair ^(1,3,4,8,13). Because dartos tissue is commonly used as an intermediate vascularized layer during urethroplasty, its biological characteristics may influence surgical outcomes, wound healing, and postoperative complications ^(1,2,4,8,14). Nevertheless, despite its clinical importance, the molecular architecture of dartos tissue remains incompletely understood.

Several histopathological studies have demonstrated abnormalities in collagen organization, elastic fibers, and connective tissue architecture in patients with hypospadias ^(8,15,16). Altered extracellular matrix composition may reduce tissue elasticity and contribute to persistent penile curvature ^(7,9,10,11,17). However, previous investigations have largely compared hypospadiac tissue with normal penile tissue or focused exclusively on histological findings ^(6,8,15). Whether molecular differences exist between ventral and dorsal dartos compartments within the same patient has received little attention ^(4,8,18).

Matrix metalloproteinase-1 (MMP-1) is a key enzyme involved in extracellular matrix turnover through degradation of fibrillar collagens, thereby maintaining connective tissue homeostasis ^(9,11,19). Reduced MMP-1 activity has been implicated in several fibrotic disorders characterized by excessive collagen accumulation and tissue stiffness ^(9,20,21). Conversely, alpha-smooth muscle actin (α -SMA) is a well-established marker of activated myofibroblasts and reflects increased contractile activity and fibrotic remodeling ^(22,23,24). Overexpression of α -SMA has been associated with pathological fibrosis in multiple organ systems and may contribute to abnormal tissue contraction ^(20,23,25).

The biological interplay between MMP-1-mediated collagen degradation and α -SMA-driven myofibroblast activation provides a plausible mechanistic framework for understanding connective tissue

abnormalities in hypospadias-associated chordee^(9,22,26). Nevertheless, compartment-specific expression patterns of these molecules within ventral and dorsal dartos tissues have not been comprehensively investigated^(8,15,18). Identifying regional molecular differences may improve our understanding of the pathogenesis of penile curvature and provide insights into tissue-specific remodeling processes relevant to reconstructive surgery^(8,11,27).

Therefore, the present study aimed to compare the gene expression profiles of MMP-1 and α -SMA between ventral and dorsal dartos tissues obtained from patients with hypospadias and chordee. We hypothesized that ventral dartos tissue exhibits reduced MMP-1 expression and increased α -SMA expression compared with dorsal tissue, reflecting localized alterations in extracellular matrix remodeling and fibrotic activity that may contribute to the development of penile curvature^(9,20,22).

Materials and Methods

Study Design and Setting

This cross-sectional observational study was conducted over a 24-month period at Arifin Achmad Regional General Hospital, Pekanbaru, Riau, Indonesia, a tertiary referral center for pediatric urology. The study aimed to evaluate differences in the gene expression of matrix metalloproteinase-1 (MMP-1) and alpha-smooth muscle actin (α -SMA) between ventral and dorsal dartos tissues in patients with hypospadias and chordee.

The study protocol was developed and reported in accordance with the principles of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement for cross-sectional studies.

Study Population

Consecutive pediatric patients diagnosed with hypospadias associated with ventral penile curvature (chordee) who underwent primary urethroplasty during the study period were screened for eligibility.

Inclusion criteria

Patients were eligible if they: (a) were between 6 months and 18 years of age; (b) had a clinical diagnosis of hypospadias associated with chordee; and (c) underwent primary surgical repair with intraoperative tissue sampling.

Exclusion criteria

Patients were excluded if they: (a) had previously undergone hypospadias repair or chordee correction; (b) had active genital or preputial infection; (c) presented with disorders of sex development or

ambiguous genitalia; (d) had insufficient tissue available for molecular analysis; or (e) had systemic diseases known to affect extracellular matrix metabolism, including connective tissue disorders or malignancy.

We have used the standard proportion formula to assess the required sample size, which yielded at least 31 participants; based on the expected 10% drop out, 33 patients were included in the study. An overview of the study procedures is shown in Fig. 1.

Sample Size

The minimum required sample size was calculated using the standard formula for estimation of a single population proportion. Based on the expected prevalence and allowing for a 10% dropout rate, at least 31 participants were required. A total of 33 patients fulfilling the eligibility criteria were ultimately enrolled.

Surgical Procedure and Tissue Collection

All procedures were performed by experienced pediatric urologists using standardized operative techniques.

After penile degloving, ventral dartos tissue was harvested from the periurethral region adjacent to the hypospadiac meatus, whereas dorsal dartos tissue was obtained from the dorsal preputial hood. Tissue specimens were immediately preserved in RNA stabilization solution and transported under controlled conditions for molecular analysis.

Assessment of Penile Curvature

Penile curvature was evaluated intraoperatively using an artificial erection test.

Artificial erection was induced by saline injection into the corpora cavernosa after temporary occlusion of the penile base. Curvature was measured using a sterile goniometer before and after dartos tissue release to document chordee severity objectively.

Chordee severity was classified according to institutional criteria as mild, moderate, or severe based on the measured angle of ventral curvature.

RNA Extraction and Quantitative Real-Time PCR

Total RNA was extracted from ventral and dorsal dartos tissue specimens using GENEzol™ reagent according to the manufacturer's protocol.

RNA concentration and purity were assessed spectrophotometrically before reverse transcription into complementary DNA (cDNA) using ReverTra Ace™ qPCR Master Mix (Toyobo, Osaka, Japan).

Quantitative real-time polymerase chain reaction (qRT-PCR) was performed using the MyGo Pro Real-Time PCR System (IT-IS International Ltd., United Kingdom).

Expression levels of MMP-1 and α -SMA were quantified relative to the housekeeping gene β -actin.

All reactions were performed in triplicate to ensure analytical reproducibility.

Relative gene expression was calculated using the comparative cycle threshold ($2^{-\Delta\Delta Ct}$) method and reported as fold-change values.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA).

Continuous variables were assessed for normality using the Kolmogorov–Smirnov test.

Normally distributed variables were compared using the independent-samples *t* test, whereas non-normally distributed variables were analyzed using the Mann–Whitney *U* test.

Categorical variables were summarized as frequencies and percentages.

A two-sided *p* value < 0.05 was considered statistically significant.

Ethical Considerations

The study protocol was approved by the Ethics Committee of the Faculty of Medicine, Universitas Andalas (Approval No. 503/UN.16.2/KEP-FK/2023).

Written informed consent was obtained from the parents or legal guardians of all participants before enrollment.

All procedures were conducted in accordance with the ethical principles of the Declaration of Helsinki.

Results

Patient Characteristics

A total of 33 pediatric patients with hypospadias associated with chordee were included in the study. The majority of participants were aged 11–14 years (19/33, 57.6%), while the remaining patients were aged ≤ 10 years or ≥ 15 years (14/33, 42.4%). Malay ethnicity was the most frequently represented ethnic group, accounting for 54.5% (18/33) of the study population.

The distribution of hypospadias severity was balanced across anatomical subtypes, with distal, midshaft, and proximal hypospadias each comprising 11 patients (33.3%). Similarly, chordee severity

was evenly distributed, with mild, moderate, and severe curvature each observed in 11 patients (33.3%). Baseline demographic and clinical characteristics are summarized in Table 1.

Table 1
Demographic and Clinical Characteristics of Participants

Variable	Category	n (%)
Age	11–14 years	19 (57.6)
	Others (≤ 10 & ≥ 15 yrs)	14 (42.4)
Ethnicity	Malay	18 (54.5)
	Others	15 (45.5)
Hypospadias Type	Distal	11 (33.3)
	Midshaft	11 (33.3)
	Proximal	11 (33.3)
Chordee Severity	Mild	11 (33.3)
	Moderate	11 (33.3)
	Severe	11 (33.3)

Differential Expression of MMP-1 Between Ventral and Dorsal Dartos Tissue

Relative mRNA expression analysis demonstrated a statistically significant difference in MMP-1 expression between ventral and dorsal dartos tissues.

The median fold-change value for ventral tissue was 0.565, compared with 0.609 for dorsal tissue. Statistical analysis using the Mann–Whitney U test demonstrated that this difference was significant ($p = 0.035$).

The distribution of MMP-1 expression values is illustrated in Fig. 2.

Differential Expression of α -SMA Between Ventral and Dorsal Dartos Tissue

Expression analysis of alpha-smooth muscle actin (α -SMA) also demonstrated significant regional differences between ventral and dorsal dartos tissues.

The median fold-change value was 1.242 in ventral tissue and 0.574 in dorsal tissue. This difference reached statistical significance using the Mann–Whitney U test ($p < 0.001$).

Individual expression distributions are presented in Fig. 3.

Summary of Molecular Findings

Overall, ventral dartos tissue demonstrated significantly lower MMP-1 expression and significantly higher α -SMA expression than dorsal dartos tissue.

These findings indicate distinct gene expression profiles between the two anatomical compartments in patients with hypospadias and chordee.

Discussion

Principal Findings

This study demonstrated significant regional differences in the expression of matrix metalloproteinase-1 (MMP-1) and alpha-smooth muscle actin (α -SMA) between ventral and dorsal dartos tissues in patients with hypospadias and chordee. Ventral dartos tissue exhibited significantly lower MMP-1 expression and significantly higher α -SMA expression than dorsal tissue. These findings suggest that the two anatomical compartments possess distinct molecular characteristics and may undergo different patterns of extracellular matrix remodeling ^(11,19).

To our knowledge, this study is among the first to specifically compare MMP-1 and α -SMA gene expression between ventral and dorsal dartos tissues within the same cohort of patients with hypospadias and chordee ⁽¹⁸⁾.

Comparison With Previous Studies

Previous studies investigating the pathophysiology of hypospadias have primarily focused on histological abnormalities or comparisons between hypospadiac and non-hypospadiac tissues ^(8,15,27). Histopathological analyses have demonstrated alterations in collagen organization, elastic fibers, and connective tissue architecture, suggesting that extracellular matrix abnormalities contribute to penile curvature ^(8,11,27).

However, direct molecular comparisons between ventral and dorsal dartos tissues remain limited ^(15,18). Our findings extend existing knowledge by demonstrating compartment-specific differences in genes involved in extracellular matrix remodeling. The reduced MMP-1 expression observed in ventral tissue is consistent with mechanisms described in fibrotic disorders, where impaired collagen degradation contributes to extracellular matrix accumulation and tissue stiffness ^(20,21,28).

Similarly, increased α -SMA expression in ventral dartos tissue may reflect enhanced myofibroblast activation ^(22,23,29). Because α -SMA is a recognized marker of myofibroblast differentiation and tissue contractility, elevated expression may indicate increased fibrotic remodeling within the ventral compartment ^(22,29,30).

Biological Implications

Extracellular matrix homeostasis depends on a dynamic balance between collagen synthesis and degradation^(11,12). MMP-1 plays an essential role in collagen turnover through degradation of fibrillar collagens, whereas α -SMA is associated with myofibroblast-mediated tissue contraction and fibrosis^(21,22,30).

The combined pattern observed in this study—lower MMP-1 expression together with higher α -SMA expression in ventral dartos tissue—may indicate a local microenvironment characterized by reduced extracellular matrix turnover and enhanced fibrotic activity^(7,20,24). Such alterations could theoretically contribute to decreased tissue elasticity and persistent ventral curvature^(8,11,27).

Nevertheless, because this investigation evaluated gene expression at the mRNA level only, no direct conclusions can be drawn regarding protein abundance or functional tissue behavior. Additional studies incorporating protein-level analyses and biomechanical assessments are required to confirm these biological mechanisms.

Clinical Relevance

From a surgical perspective, understanding regional molecular differences within dartos tissue may improve knowledge of tissue biology during hypospadias reconstruction^(4,8,31).

Although dorsal dartos flaps are commonly used as vascularized coverage during urethroplasty, the present study was not designed to evaluate surgical outcomes or determine whether molecular differences influence postoperative complications^(1,3,4,12). Therefore, the observed expression patterns should be interpreted as hypothesis-generating findings rather than direct evidence supporting a specific operative technique.

Future prospective studies correlating molecular markers with surgical outcomes may clarify whether these biological differences have practical implications for reconstructive decision-making.

Strengths and Limitations

The principal strength of this study lies in the paired comparison of ventral and dorsal dartos tissues obtained from the same patients, thereby minimizing interindividual biological variability.

However, several limitations should be acknowledged. First, the study included a relatively small sample from a single institution, which may limit external validity. Second, molecular analysis was restricted to mRNA expression without confirmation at the protein level using immunohistochemistry or Western blot analysis. Third, no comparison group consisting of patients without chordee was available, making it difficult to determine whether the observed differences are specific to penile curvature or represent intrinsic regional variation. Finally, functional biomechanical assessment of tissue elasticity was not performed^(8,15).

Future Directions

Future multicenter studies with larger sample sizes should incorporate protein expression analyses, histological correlation, and functional biomechanical testing to validate the present findings. Inclusion of comparison groups without chordee and longitudinal evaluation of postoperative outcomes may further clarify the role of extracellular matrix remodeling in the pathogenesis of hypospadias-associated penile curvature ^(8,11,32).

Overall Interpretation

In conclusion, the present findings provide preliminary evidence that ventral and dorsal dartos tissues exhibit distinct molecular expression profiles with respect to MMP-1 and α -SMA. These regional differences may reflect localized variation in extracellular matrix remodeling and fibrotic activity ^(11,20,21). Although the clinical significance of these findings remains to be established, they contribute to the growing understanding of connective tissue biology in hypospadias and provide a foundation for future translational research ^(8,15,18).

Conclusion

This study demonstrated significant differences in the expression of matrix metalloproteinase-1 (MMP-1) and alpha-smooth muscle actin (α -SMA) between ventral and dorsal dartos tissues in patients with hypospadias and chordee. Ventral dartos tissue exhibited lower MMP-1 expression and higher α -SMA expression than dorsal tissue, suggesting regional variation in extracellular matrix remodeling and fibrotic activity.

These findings provide preliminary molecular evidence that ventral and dorsal dartos tissues are biologically distinct and may contribute differently to the pathophysiology of penile curvature. However, because the present study evaluated gene expression at the mRNA level only, further investigations incorporating protein-level validation, histological correlation, and functional tissue assessment are required before definitive biological or clinical conclusions can be drawn.

Future multicenter studies with larger patient populations and long-term clinical follow-up are warranted to determine whether these molecular differences have implications for surgical reconstruction and postoperative outcomes in patients with hypospadias and chordee.

Abbreviations

α -SMA

Alpha-smooth muscle actin

cDNA

Complementary deoxyribonucleic acid

ECM

Extracellular matrix

MMP-1

Matrix metalloproteinase-1

mRNA

Messenger ribonucleic acid

qRT-PCR

Quantitative real-time polymerase chain reaction

TIP

Tubularized incised plate

Declarations

Ethics Approval and Consent to Participate

The study protocol was approved by the Ethics Committee of the Faculty of Medicine, Universitas Andalas, Indonesia (Approval No. 503/UN.16.2/KEP-FK/2023).

Consent for Publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

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Author Contribution

Authors' Contributions
JP (Joko Pitoyo): Conceptualization, methodology, investigation, patient recruitment, surgical procedures, data curation, formal analysis, visualization, and writing – original draft.
A (Alvarino): Conceptualization, methodology, supervision, validation, interpretation of data, critical revision of the manuscript, and writing – review & editing. Alvarino also served as the corresponding author.
AF (Afdal): Methodology, supervision, validation, interpretation of molecular data, and writing – review & editing.
IJ (Indra Jaya): Formal analysis, statistical analysis, methodology, data interpretation, and writing – review & editing.
MAY (Muhammad Adan Yashar): Investigation, laboratory coordination, data curation, and writing – review & editing.
NADP (Naura Alifa Daniesya Pitoyo): Literature review, data

curation, visualization, manuscript preparation, and writing – review & editing. All authors read and approved the final manuscript and agree to be accountable for all aspects of the work.

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Availability of Data and Materials

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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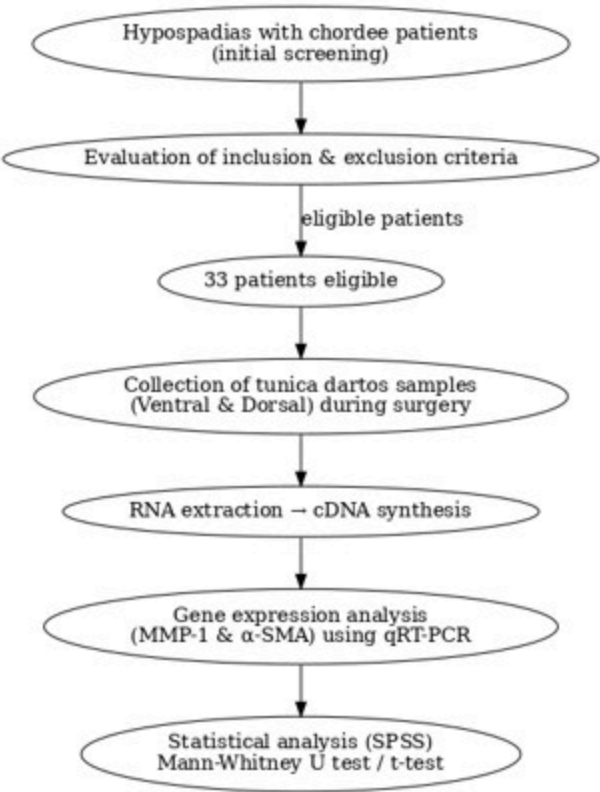
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Figures

Figure 1. Clinical Research Flowchart of Hypospadias with Chordee



Note: This flowchart illustrates the research process, from patient selection to statistical analysis.

Figure 1

Clinical Research Flowchart.

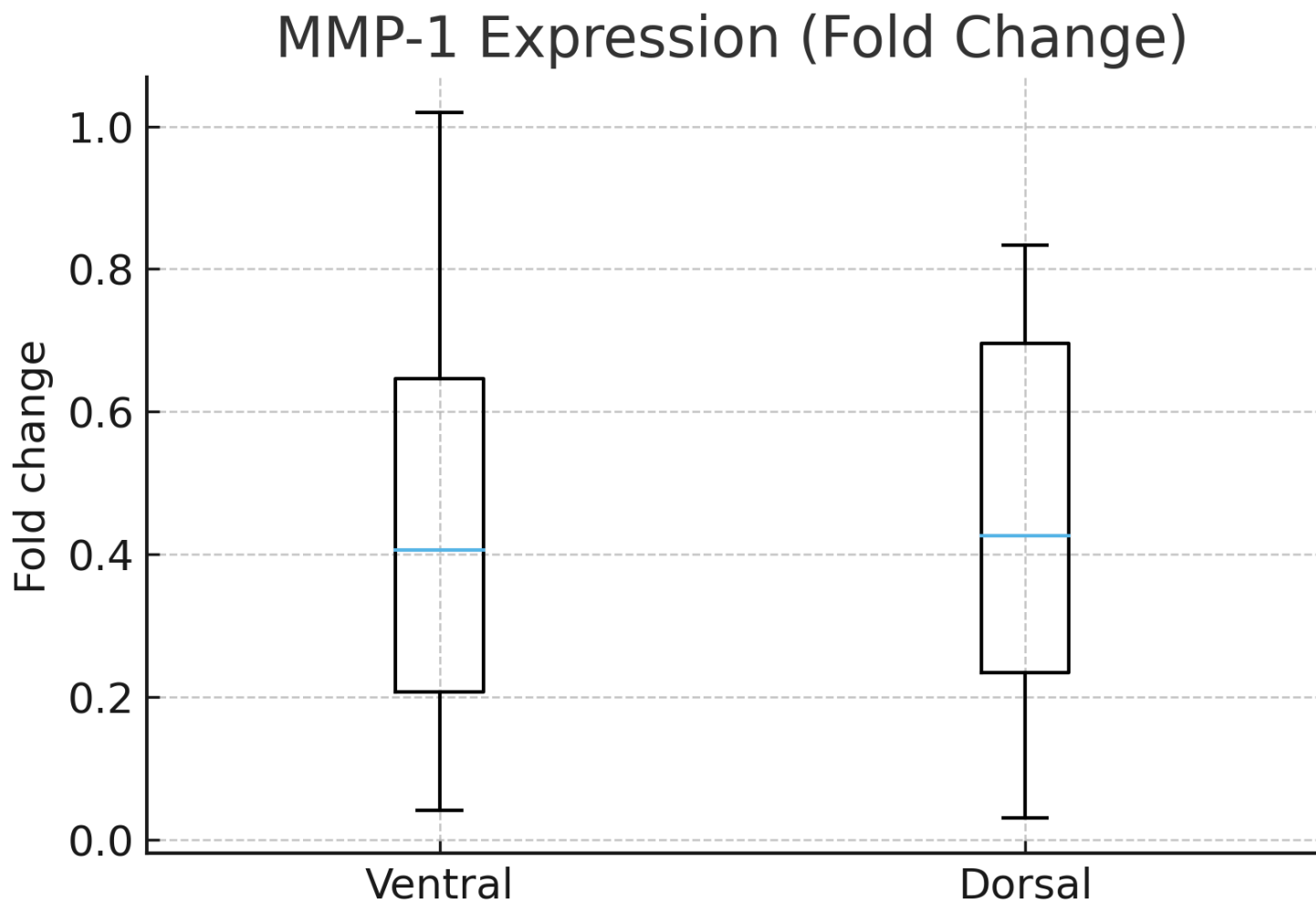


Figure 2

Expression of MMP-1 in ventral versus dorsal tunica dartos (boxplot).

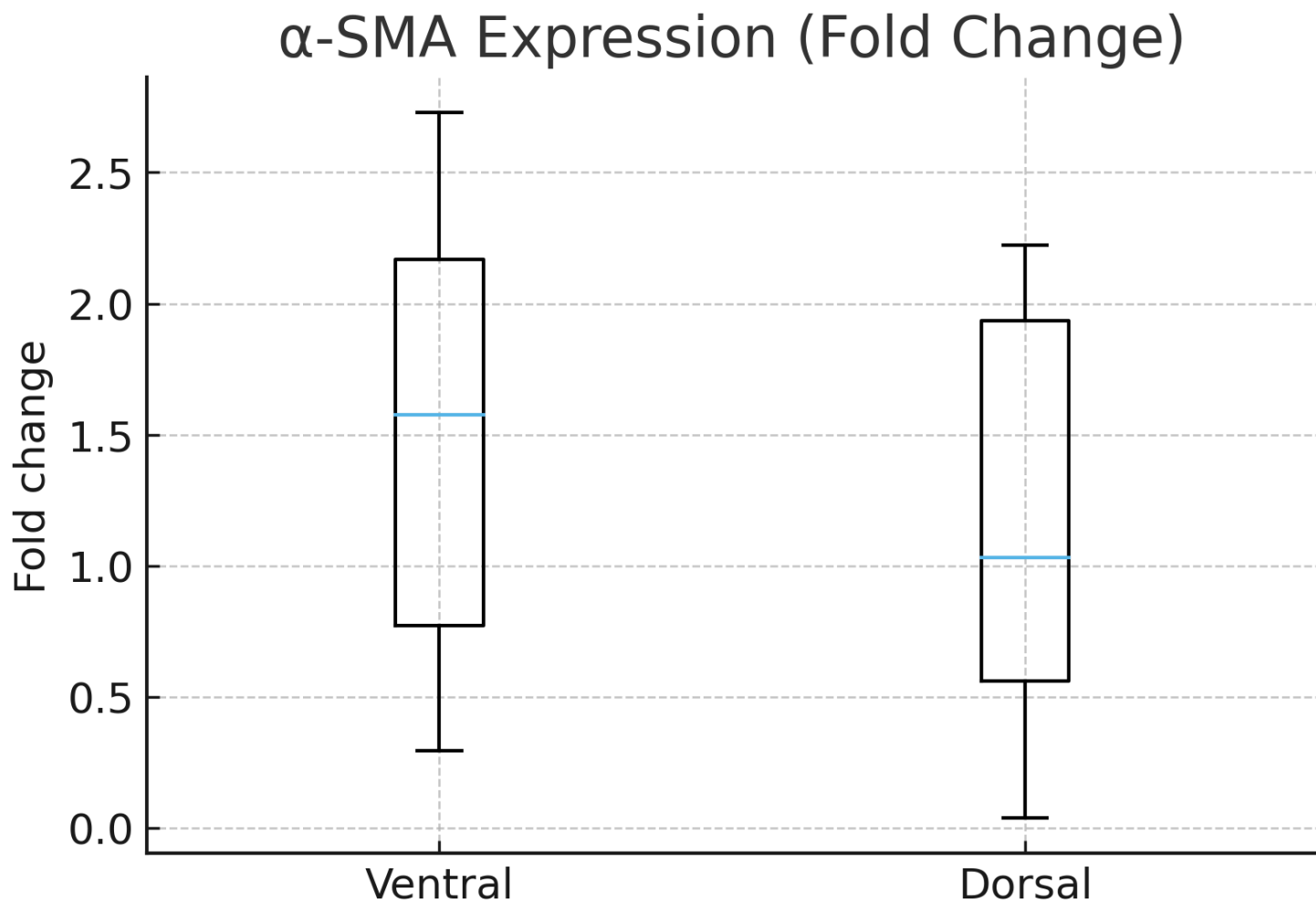


Figure 3

Expression of α -SMA in ventral versus dorsal tunica dartos (boxplot).