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Comparative immunohistochemical expression of α-smooth muscle actin in pediatric indirect inguinal hernia and hydrocele

Perbandingan ekspresi imunohistokimia α-smooth muscle actin (SMA) pada hernia inguinal indirek dan hidrokel anak

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Abstract

Background: Indirect inguinal hernia and hydrocele, with reported incidences of up to 4% and 0.0034%, respectively, are consequences of incomplete obliteration of the processus vaginalis. Although both conditions share a similar embryological origin, the molecular mechanisms driving their distinct clinical presentations remain unclear. Alpha-smooth muscle actin (α-SMA) is a widely used marker of myofibroblast activity and tissue remodeling. This study aimed to compare the expression of α-SMA in the processus vaginalis tissue of pediatric patients with indirect inguinal hernia and hydrocele.

Methods: An observational analytical study with a cross-sectional approach was conducted at the H. Adam Malik General Hospital between January and June 2025. Processus vaginalis tissues were collected from 50 male pediatric patients (25 hernia and 25 hydrocele cases). The expression of α-SMA was evaluated through semi-quantitative immunohistochemical analysis, assessing both staining intensity and distribution. Data were analyzed using the chi-square test, and $p < 0.05$ was considered statistically significant.

Results: A total of 50 patients were enrolled in the study, with a mean age of 3.98 ± 3.78 y. Statistical analysis revealed a highly significant difference in α-SMA expression between the two groups ($p < 0.001$). Strong α-SMA expression was observed in 96% of the indirect inguinal hernia group, whereas 96% of the hydrocele group exhibited weak expression (OR 576; 95% CI 34.0–9751.0; $p < 0.001$). The magnitude of this association indicates a strong relationship between clinical diagnosis and α-SMA expression.

Conclusion:

α-SMA expression differed significantly between indirect inguinal hernias and hydroceles, with a strong expression predominating in hernia specimens. These findings support an association between indirect inguinal hernias and increased α-SMA–positive stromal elements in the processus vaginalis.

Keywords:

α-SMA, Immunohistochemistry, Pediatric Surgery

Abstrak

Latar Belakang: Hernia inguinalis indirek dan hidrokel, dengan insidensi yang dilaporkan masing-masing hingga 4% dan 0,0034%, merupakan konsekuensi dari obliterasi prosesus vaginalis yang tidak sempurna. Meskipun kedua kondisi memiliki asal embriologis yang serupa, mekanisme molekuler yang mendasari perbedaan manifestasi klinisnya masih dalam penelitian. Alpha-smooth muscle actin (α-SMA) merupakan penanda yang banyak digunakan untuk menilai aktivitas miofibroblas dan proses remodeling jaringan. Penelitian ini bertujuan untuk membandingkan ekspresi α-SMA pada jaringan prosesus vaginalis pasien anak dengan hernia inguinalis indirek dan hidrokel.

Metode: Penelitian analitik observasional dengan pendekatan potong lintang dilakukan di RSUP H. Adam Malik pada Januari hingga Juni 2025. Jaringan prosesus vaginalis dikumpulkan dari 50 pasien anak laki-laki (25 kasus hernia dan 25

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kasus hidrokel). Ekspresi α-SMA dievaluasi melalui analisis imunohistokimia semi-kuantitatif dengan menilai intensitas dan distribusi pewarnaan. Data dianalisis menggunakan uji Chi-square, dengan p < 0,05 dianggap bermakna secara statistik.
Hasil: Sebanyak 50 pasien diikutsertakan dalam penelitian dengan rerata usia 3,98 ± 3,78 tahun. Analisis statistik menunjukkan perbedaan yang sangat bermakna pada ekspresi α-SMA antara kedua kelompok (p < 0,001). Ekspresi α-SMA kuat ditemukan pada 96% kelompok hernia inguinalis indirek, sedangkan 96% kelompok hidrokel menunjukkan ekspresi lemah (OR 576; IK 95% 34,0–9751,0; p < 0,001). Nilai odds ratio tersebut mencerminkan kekuatan asosiasi yang tinggi antara hernia inguinalis indirek dan ekspresi α-SMA yang kuat.
Kesimpulan: Ekspresi α-SMA berbeda secara signifikan antara hernia inguinalis indirek dan hidrokel, dengan ekspresi kuat predominan pada spesimen hernia. Temuan ini mendukung adanya asosiasi antara hernia inguinalis indirek dan peningkatan elemen stroma positif α-SMA pada prosesus vaginalis.

Kata Kunci:
α-SMA, Imunohistokimia, Bedah Anak

Introduction

Indirect inguinal hernia and hydrocele in children are common manifestations of patent processus vaginalis (PPV), an embryologic peritoneal evagination that normally closes after testicular descent. Pediatric indirect inguinal hernia has been reported in approximately 0.8%–4% of infants, and hydrocele is frequently encountered during infancy and childhood (Kummari et al., 2024; Öberg et al., 2017; Salari et al., 2025). Although both conditions share a similar embryologic origin, the tissue characteristics associated with persistent patency appear to differ between the clinical entities. Prior histopathological studies have suggested that the persistence and phenotype of smooth muscle–related cells within the processus vaginalis may vary between hernia and hydrocele sacs, and immunohistochemistry has been used to characterize these differences (Krishnan et al., 2024).

Alpha-smooth muscle actin (α-SMA) is widely used as an immunohistochemical marker for contractile stromal cells, including myofibroblast-like elements involved in tissue remodeling. However, important uncertainties remain in the international literature regarding how consistently α-SMA expression patterns distinguish pediatric indirect inguinal hernia from hydrocele across populations and clinical settings and whether these patterns are comparable across centers using semi-quantitative scoring (Krishnan et al., 2024).

In Indonesia, comparative immunohistochemical data on α-SMA expression in the processus vaginalis of children with indirect inguinal hernia versus hydrocele remain limited. Therefore, this study aimed to compare semi-quantitative α-SMA immunohistochemical expression in processus vaginalis tissue of pediatric patients with indirect inguinal hernia and hydrocele treated at H. Adam Malik General Hospital, Medan, Indonesia. We hypothesized that the α-SMA expression categories (weak vs. strong) differ between these two clinical entities.

Methods

Study Design and Setting

This study employed an analytical observational design with a cross-sectional approach to compare the α-SMA expression in pediatric patients with indirect inguinal hernia and hydrocele. The research was conducted at H. Adam Malik General Hospital, Medan, Indonesia, between January and June of 2025. This facility was selected because it serves as the primary tertiary referral center in North Sumatra, with adequate pediatric surgery and anatomical pathology laboratory support for immunohistochemical analysis. Ethical approval was obtained from the Institutional Review Board of Universitas Sumatera Utara and H. Adam Malik Central General Hospital (Approval No: 1388/KEPK/USU/2025).

Participants and Sampling

The target population comprised children diagnosed with indirect inguinal hernias and hydroceles who underwent elective surgery. The sample size was calculated using the two-proportion comparison formula based on previous data from Krishnan et al. as follows:

$$n = \frac{\left\{Z_{1-\frac{\alpha}{2}}\sqrt{(2P(1-P))} + Z_{1-\beta}\sqrt{(P1(1-P1)) + P2(1-P2)}\right\}^2}{(P1-P2)^2}$$

$$n = \frac{\left\{1.96\sqrt{(2 \times 0.345 \times 0.655)} + 0.842\sqrt{0.53 \times 0.47 + 0.16 \times 0.84}\right\}^2}{(0.53-0.16)^2}$$

$$n = \frac{\{1.96 \times 0.6721 + 0.842 \times 0.6192\}^2}{(0.37)^2}$$

$$n = \frac{\{1.3163 + 0.5209\}^2}{0.1369}$$

$$n = \frac{3.3733}{0.1369} \approx 24.64$$

Assuming alpha = 0.05 and statistical power = 80%, a minimum of 25 subjects per group was required. Participants were recruited using consecutive sampling during the study period. Inclusion and exclusion criteria were strictly applied; the subjects were boys aged 1–18 years with viable processus vaginalis tissue. Patients with a history of scrotal infection, prior inguinal surgery, or systemic congenital syndromes were excluded from the study. Written informed consent was obtained from the parents or legal guardians prior to the tissue collection.

Data Collection and Immunohistochemistry

Processus vaginalis tissue specimens were obtained intraoperatively from pediatric patients undergoing elective surgery for indirect inguinal hernias or hydrocele. Immediately after excision, the tissue samples were fixed in 10% neutral-buffered formalin for 24–48 h and subsequently processed using standard paraffin-embedding procedures. Serial sections (3–4 µm thick) were prepared and mounted on poly L-lysine-coated glass slides for immunohistochemical analysis.

Immunohistochemical staining was performed using a rabbit polyclonal anti-α-smooth muscle actin (α-SMA) antibody (Bioss Antibodies, Beijing, China; catalog no. bs-0189R). Paraffin-embedded sections (3–4 µm) were deparaffinized in xylene and rehydrated with graded ethanol solutions. Antigen retrieval was performed using heat-induced epitope retrieval in a citrate buffer (pH 6.0). Endogenous peroxidase activity was blocked with 3% hydrogen peroxide.

The primary antibody was applied at a dilution of 1:100 for immunohistochemistry on paraffin sections (IHC-P 1:100), followed by incubation with an appropriate secondary antibody using a standard streptavidin–biotin detection system. Immunoreactivity was visualized using 3,3′-diaminobenzidine (DAB) chromogen, and the slides were counterstained with hematoxylin.

The positive control sections consisted of smooth muscle–rich tissue known to express α-SMA, whereas the negative controls were processed identically with the omission of the primary antibody to confirm staining specificity.

All slides were evaluated by a single, experienced anatomical pathologist who was blinded to the clinical diagnosis. Inter-observer reliability testing was not conducted.

α-SMA expression was evaluated using a light microscope by an experienced anatomical pathologist blinded to the clinical diagnosis. Expression was assessed semi-quantitatively by multiplying the staining intensity (score 1–4) by the percentage distribution of positive cells (score 1–4). The final immunoreactivity scores ranged from 1–16. A total score of ≤4 was categorized as weak expression, whereas a score of >4 was categorized as a strong expression.

Statistical Analysis

The data were systematically processed using a statistical software. Normality testing was performed using the Shapiro–Wilk test. Baseline characteristics between groups were compared using the independent t-test or the Mann–Whitney U test, as appropriate. Categorical variables, including the comparison of α-SMA expression intensities between the hernia and hydrocele groups, were analyzed using the chi-square test or Fisher's exact test as appropriate. Demographic characteristics were presented descriptively. Statistical significance was set at $P < 0.05$. All statistical analyses were performed using SPSS version 25.0.

Results

A total of 50 pediatric patients were included in this study, consisting of 25 (50%) with indirect inguinal hernia and 25 (50%) with hydroceles. The demographic characteristics and clinical distribution of participants are presented in Table 1. All subjects were male, with a mean age of 3.98 ± 3.78 years. The right side was the most frequent site of clinical manifestation (62%). No. Statistically significant differences were observed in the baseline demographic characteristics of participants.

Table 1. Demographic characteristics of participants.

Variable	Mean ± SD (Min-Max)		p-value
Age (years)	3.98 ± 3.78 (1 – 14)		0.91[a]
Duration of symptoms (months)	5.6 ± 7.2 (1 – 36)		0.84[a]
Variable	f	%	p-value
Gender			-
Male	50	100	
Female	0	0	
Clinical Diagnosis			-
Indirect Inguinal Hernia	25	50	
Hydrocele	25	50	
Side of Defect			0.88[b]
Right	31	62	
Left	16	32	
Bilateral	3	6	

Immunohistochemical analysis revealed a significant difference in the expression of alpha-smooth muscle actin between the two groups (Table 2). In the hydrocele group, 96% of the cases showed weak expression, whereas in the indirect inguinal hernia group, 96% of the cases exhibited strong expression. Statistical analysis yielded a p-value of <0.001, indicating a highly significant difference in the expression levels between the clinical entities.

Table 2. Comparison of α-smooth muscle actin expression between groups.

Variable	A-SMA Expression		OR (95% CI)	p-value
	Weak (n, %)	Strong (n, %)		
Hydrocele	24 (96%)	1 (4%)	Reference	<0.001
Indirect Inguinal Hernia	1 (4%)	24 (96%)	576 (34.0–9751.0)	

The odds of exhibiting strong α-SMA expression were substantially higher in the indirect inguinal hernia group than in the hydrocele group (OR 576; 95% CI 34.0–9751.0), indicating a strong association between diagnosis and immunohistochemical expression patterns.

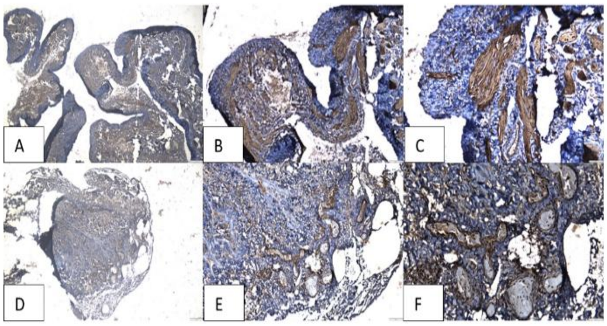

Figure 1. Histopathology of Research Samples. (A) 40x Indirect Inguinal Hernia; (B) 100x Indirect Inguinal Hernia; (C) 200x Indirect Inguinal Hernia; (D) 40x Hydrocele; (E) 100x Hydrocele; (F) 200x Hydrocele.

Histopathological examination at different magnifications revealed variations in the staining intensity and cellular distribution of α-SMA in the processus vaginalis tissue. In indirect inguinal hernia samples, the staining was markedly more intense and diffuse than the minimal and localized staining observed in the hydrocele samples (Figure 1).

Discussion

This study identified a significant disparity in α-SMA expression between indirect inguinal hernias and hydroceles in pediatric patients. Indirect inguinal hernia, the most common form of indirect hernia in children, arises from the failed obliteration of the processus vaginalis following testicular descent, creating a persistent communication between the peritoneal cavity and inguinal canal (Chen et al., 2017; Patoulias et al., 2020; Somuncu & Somuncu, 2021). Our data align with global epidemiological trends, showing a marked male predominance (100%) and right-sided lateralization (62%). This phenomenon is likely attributed to the delayed descent of the right testis and distinct vascular anatomical configurations (Tanyel, 2024).

From a histopathological perspective, α-SMA is a primary marker of myofibroblast activity, which is essential for tissue contraction and remodeling. The elevation in the expression of α-SMA in 96% of hernia tissues indicates that the stromal components within hernia sacs exhibit greater α-SMA–positive staining than those within hydrocele sacs. In contrast, 96% of hydrocele tissues exhibited weak expression ($p < 0,001$), suggesting differences in the cellular composition of the processus vaginalis tissue between the two groups. Importantly, the present study was designed to evaluate immunohistochemical expression patterns rather than to investigate molecular pathways; therefore, interpretation is limited to observed differences in staining intensity and distribution.(Amato et al., 2022).

Our findings are consistent with those of a previous review by Tanyel, which stated that smooth muscle cell and myofibroblast differentiation, marked by α-SMA expression, plays a pivotal role in the failed obliteration of the processus vaginalis (Tanyel, 2024). Similarly, Krishnan et al. indicated that α-SMA expression is significantly higher in hernia and hydrocele tissues than in undescended testes, reinforcing the role of smooth muscle cells in the pathogenesis of these conditions (Krishnan et al., 2024). Although some studies suggest that α-SMA expression can vary based on patient age or tissue thickness, this study solidifies the evidence that higher α-SMA levels are closely associated with the active pathogenesis of indirect inguinal hernias (Ramos et al., 2019).

These findings have clinical implications, as α-SMA expression could serve as a potential indicator of myofibroblast activity, which the authors hypothesized may be associated with postoperative recurrence risk.

The pathogenesis of indirect inguinal hernias is multifactorial and remains poorly understood. Although congenital factors play a central role, variability in tissue characteristics may contribute to differences in clinical presentation. The present findings should be interpreted within this broader context, without implying specific molecular mechanisms, as the current study was limited to immunohistochemical assessment of α-SMA expression.

Compared with prior studies, our study adds region-specific data using a standardized semi-quantitative scoring system and uniform laboratory procedures within a single tertiary referral center. While previous studies have examined smooth muscle–related markers in the processus vaginalis tissue, direct comparisons between pediatric indirect inguinal hernia and hydrocele using a consistent immunohistochemical scoring framework remain limited. The present results contribute to this area by demonstrating a marked difference in α-SMA expression patterns between the two conditions within a defined pediatric population.

Several limitations of this study should be considered when interpreting the findings. The sample size was relatively small, which may have affected the stability of the estimates and limited the extent to which the results could be applied to other pediatric populations. In addition, the study was conducted at a single tertiary referral center; therefore, the findings may not fully reflect the patterns observed in different hospitals or regions. The assessment of α-SMA expression relied on a semi-quantitative immunohistochemical scoring system, which, although based on predefined criteria, still involved some degree of observer's interpretation. Molecular validation methods such as western blotting or reverse transcription polymerase chain reaction (RT-PCR) were not performed; therefore, quantitative confirmation of protein expression was not possible. Multivariate analysis was not performed primarily because the sample size was limited and the distribution of expression categories showed near-complete separation between groups, making regression modeling unreliable. Future studies with larger multicenter samples, objective digital quantification of staining, and complementary molecular analyses will help to strengthen and confirm these findings.

Conclusion

In conclusion, this study demonstrated a significant disparity in α-SMA expression between indirect inguinal hernias and hydroceles, where hernia cases predominantly showed strong expression compared to the weak expression observed in the majority of hydrocele cases. These findings indicate that although both conditions are associated with a patent processus vaginalis, their stromal expression patterns are not identical. Further studies are required to validate these results in a broader population.

Based on these findings, α-SMA immunohistochemical examination can be considered a supporting tool for evaluating the tissue characteristics of the processus vaginalis in cases of indirect inguinal hernia in children. This examination has the potential to aid in understanding local pathogenesis patterns and serve as a basis for further research related to the risk of recurrence and more targeted management strategies in tertiary care centers.

Conflict Interest

The authors declare no conflicts of interest

References

Amato, G., Romano, G., Rodolico, V., Puleio, R., Calò, P. G., Di Buono, G., Cicero, L., Romano, G., Goetze, T. O., & Agrusa, A. (2022). Dynamic Responsive Inguinal Scaffold Activates Myogenic Growth Factors Finalizing the Regeneration of the Herniated Groin. *Journal of Functional Biomaterials*, *13*(4). https://doi.org/10.3390/jfb13040253

Baldini, E., Sorrenti, S., Lori, E., Palla, L., Cardarelli, S., Pironi, D., Tripodi, D., Pavan, A., Fakeri, A., Cobo, V., Pellegrini, C., Nardi, P., Rinaldi, V., Ulisse, S., & Palumbo, P. (2025). Circulating Biomarkers as Potential Risk Factors for Inguinal Hernia. *International Journal of Molecular Sciences*, *26*(15), 1–15. https://doi.org/10.3390/ijms26157032

Chen, J., Chu, C., Shen, Y., Zou, Z., & Yuan, X. (2017). Individualized Treatment of Inguinal Hernia in Children. In *Hernia*. IntechOpen. https://doi.org/10.5772/intechopen.68875

Correia, M. L. A., Filho, F. M. P., Júnior, S. C. G., & Peixoto, M. V. M. (2023). Effects of intra-abdominal hypertension on maternal-fetal outcomes in term pregnant women: A systematic review. *PLoS ONE*, *18*(6 JUNE), 1–13. https://doi.org/10.1371/journal.pone.0280869

Krishnan, N., Kakkar, A., Nag, T. C., Agarwala, S., Goel, P., & Dhua, A. K. (2024). A comparative study of smooth muscle cell characteristics and myofibroblasts in processus vaginalis of pediatric inguinal hernia, hydrocele and undescended testis. *BMC Urology*, *24*(1), 115. https://doi.org/10.1186/s12894-024-01449-0

Kummari, S., Subburam, S., & Chokkalingam, S. R. (2024). An Indirect Inguinal Hernia in a Neonate Containing the Uterus, Ovary, and Fallopian Tube: A Report of a Rare Case and a Literature Review. *Cureus*, *16*(7), e65440. https://doi.org/10.7759/cureus.65440

Öberg, S., Andresen, K., & Rosenberg, J. (2017). Etiology of inguinal hernias: a comprehensive review. *Frontiers in Surgery*, *4*, 52.

Patoulias, I., Koutsogiannis, E., Panopoulos, I., Michou, P., Feidantsis, T., & Patoulias, D. (2020). Hydrocele in Pediatric Population. *Acta Medica*, *63*(2), 57–62. https://doi.org/10.14712/18059694.2020.17

Puri, P., Friedmacher, F., Farrugia, M.-K., Sharma, S., Esposito, C., & Mattoo, T. K. (2024). Primary vesicoureteral reflux. *Nature Reviews Disease Primers*, *10*(1), 75. https://doi.org/10.1038/s41572-024-00560-8

Ramos, D. M., d'Ydewalle, C., Gabbeta, V., Dakka, A., Klein, S. K., Norris, D. A., Matson, J., Taylor, S. J., Zaworski, P. G., & Prior, T. W. (2019). Age-dependent SMN expression in disease-relevant tissue and implications for SMA treatment. *The Journal of Clinical Investigation*, *129*(11), 4817–4831.

Salari, N., Beiromvand, M., Abdollahi, R., Hemmatabadi, F. K., Daneshkhah, A., Ghaderi, A., Asgari, M., & Mohammadi, M. (2025). Global prevalence of hydrocele in infants and children: a systematic review and meta-analysis. *BMC Pediatrics*, *25*(1), 128. https://doi.org/10.1186/s12887-025-05492-0

Somuncu, S., & Somuncu, Ö. S. (2021). A Comprehensive Review: Molecular and Genetic Background of Indirect Inguinal Hernias. *Visceral Medicine*, *37*(5), 349–357. https://doi.org/10.1159/000515275

Tanyel, F. C. (2024). Obliteration of the Processus Vaginalis After Testicular Descent. *Balkan Medical Journal*, *41*(2), 89–96. https://doi.org/10.4274/balkanmedj.galenos.2024.2023-12-111

Zhang, W., Guo, Z., Li, L., Shi, Z., & Zhu, T. (2022). Hypoxia Promotes Human Umbilical Vein Smooth Muscle Cell Phenotypic Switching via the ERK 1/2/c-fos/NF-κB Signaling Pathway. *Annals of Vascular Surgery*, *84*, 371–380. https://doi.org/10.1016/j.avsg.2022.03.038