BMC Urology

RESEARCH

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Expression of mRNA vascular endothelial growth factor in hypospadias patients

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Abstract

Background: Hypospadias is a relatively common genital anomaly in humans, usually followed by inelastic dartos that causes penile chordee. Vascular endothelial growth factor (*VEGF*) is strongly linked to the viscoelasticity of tissues and their elastic phase. This study aimed to evaluate *VEGF* expressions in (1) fascia dartos between hypospadias and controls and (2) chordee severity.

Methods: This prospective cohort study involved 65 specimens from patients with hypospadias and ten specimens from controls. The samples were analyzed by quantitative real-time polymerase chain reaction (qPCR) for *VEGF* expression.

Results: The expressions of *VEGF* were not different between proximal and distal hypospadias patients and controls (fold change: distal − 0.25; fold change: proximal − 0.2; $p = 0.664$). The scaled expressions related to chordee severity were mild − 0.1; moderate 0.1; severe − 0.25 ($p = 0.660$).

Conclusions: *VEGF* expressions might not affect the severity of hypospadias and chordee, implying the pathogenesis is complex involving many growth factors. Further study with a larger sample size is necessary to clarify and confirm our findings.

Keywords: Hypospadias, *VEGF*, Dartos tissue, Elasticity, Chordee, Penile curvature

Introduction

Hypospadias, a relatively common human genital abnormality, is a urethral opening disorder that is not positioned correctly at the tip of the penis. Hypospadias is the second most common congenital anomaly in boys and the most common type of penis deformity [1, 2]. Molecular processes are needed in the occurrence of hypospadias, but the relationships and functions of these two elements are still unknown [3]. The exact cause of hypospadias has been identified in about 20% of cases, especially in the most challenging conditions [1].

Hypospadias may be related to an abnormality of the dartos fascia. However, this hypothesis is not supported by solid evidence from histopathology. There are contrasting studies of histopathological aspects of connective tissue in patients with hypospadias. The dartos fascia of hypospadias had abnormal tissue that gave it the thick and inelastic character [4, 5]. Inelastic dartos fascia tissue in patients diagnosed with hypospadias is a pathological tissue [4, 6, 7].

VEGF regulates elastic modulus and increases matrix stiffness in cardiomyocytes [8]. It is strongly associated with tissue viscoelasticity and the elastic cervix phase shortening [9]. *In addition, VEGF* plays a pivotal part in endothelial cells' proliferation, migration, and differentiation [10]. *VEGF* induces α1β1 and α2β1 integrins expression in microvascular endothelial cells [11], endothelial cell migration, and proliferation [12, 13]. *VEGF* is not retained intra-cellularly but attaches to the cell surface or

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extracellular membrane (ECM) and other matrix metalloproteinases (MMPs) [14]. However, the role of *VEGF* on dartos tissue remains unknown. Hence, this study aimed to investigate the impact of *VEGF* modulated dartos on tissue elasticity.

Methods

Patients

A prospective cohort study was performed in 65 patients who underwent repair of hypospadias with chordee without DSD, and ten patients underwent circumcision as controls between 2018 and 2020. The samples were then divided into three groups: distal hypospadias, proximal hypospadias, and controls. We used periurethral dartos tissues collected during excision of chordee. The Ethical Committee of our university approved this study(KE/0287/03/2020), and written informed consent to participate in this study was obtained from all the parents.

qPCR analysis

All dartos tissue of each patient was placed in a single tube. All samples were homogenized. Total RNA was extracted using Hybrid-R™ Isolation Kit, and cDNA was extracted from 200 ng of total RNA using NEXpro™ qRT-PCR Kit. Gene expression analysis by Quantitative PCR has been used for *VEGF* (F: 5′-CCCACTGAG GAGTCCAACAT-3′ and R: 5′-AAATGCTTTCTCCGC TCTGA-3′), and GAPDH (F: 5′-GCATCCTGGGCT ACACTGAG-3′ and R: 5′-TCCACCACCCTGTTGCTG TA-3′) used as internal control. Conditions for amplification consisted of an initial denaturing phase at 95 °C for 10 min, proceeded by 40 cycles at 95 °C for 20 secs, at 56 °C for 40 secs, and 72 °C for 60 secs. Extension done at 72 °C for 5 min. The qPCR amplified samples were analyzed by the BiONEER Exicycle™ 96 (BioNEER, Daejeon, South Korea). The Livak method was used to measure fold change between the three groups [15].

Statistical analysis

The Kruskal–Wallis test was used to compare VEGF expressions in control, distal and proximal hypospadias' tissues. Post hoc analysis used Mann–Whitney U test for comparison of each group. Values were considered statistically significant with $p < 0.05$.

Results

The age mean was 6.24 ± 4.36 years. Our study consisted of 24 (32%) distal hypospadias, 41 (54.7%) proximal hypospadias, and ten (13.3%) controls. Furthermore, five (6.7%) patients had penoscrotal transposition, and 70 (93.3%) patients were without penoscrotal transposition. Penile curvature in hypospadias was grouped into four groups: mild (< 30 degrees), moderate (30–60 degree), severe (> 60 degrees), and normal with each group number respectively: 19 (25.3%), 15 (20%), 23 (30.7%) and 18 patients (24%) (Table 1).

The qPCR analyses showed decreasing of *VEGF* expression in both distal and proximal (− 1.05 (− 0.1–(− 1.9)); − 1 (− 0.2–(− 2.3))) hypospadias compared with the control group − 0.8 (− 0.4–(− 1.1)), however were not significantly different. The fold change of VEGF expression in proximal and distal hypospadias and controls was not significantly different (mean fold change: hypospadias distal − 0.25; mean fold change: hypospadias proximal − 0.2; $p = 0.664$). Meanwhile, fold change expressions of *VEGF*-related chordees were scaled as follows: (mild − 0.1; moderate 0.1; severe − 0.25; $p = 0.660$) (Table 2).

Discussion

In almost every organ, the biomechanical properties of connective tissues perform essential physiological roles. Biochemical and biophysical properties of ECM are responsible for migration, adhesion, the integrity of

Table 1 Clinical characteristics of patients in our institution

Variable	
All groups (n = 75)	
Age, yr. # mean	6.24 ± 4.36
VEGF, Median (min–max) *	− 0.9 (− 0.1–(− 2.3)
Hypospadias Patients (n = 65)	
Hypospadias Type, n (%)	
Distal	24 (32)
Glandular	5 (20.8)
Subcoronal	5 (20.8)
Midshaft	14 (58.4)
Proximal	41 (54.7)
Penoscrotal	27 (65.)
Scrotal	13 (31.7)
Perineal	1 (2.4)
Penile curvature, n (%)	
Mild (< 30 degree)	12 (16)
Moderate (30–60 degree)	15 (20)
Severe (> 60 degree)	38 (50.7)
Normal	10 (13.3)
Penoscrotal transposition	
Yes	7 (9.3)
No	68 (90.7)
Bifid Scrotum	
Yes	7 (9.3)
No	68 (90.7)

VEGF, vascular endothelial growth factor

*Kolmogorov–Smirnov $p > 0.05$

Kolmogorov–Smirnov $p > 0.05$

Table 2 Analysis based on hypospadias type and penile curvature severity

Variable	VEGF[#]	Fold change	*p*
Hypospadias type			
Distal hypospadias	− 1.05 (− 0.1–(− 1.9))	3.07	0.664
Proximal hypospadias	− 1 (− 0.2–(− 2.3))	2.96	
Control	− 0.8 (− 0.4–(− 1.1))		
Penile curvature severity			
Mild	− 0.9 (− 0.2–(− 1.4))	4.60	0.660
Moderate	− 0.7(− 0.1–(− 1.6))	0.723	
Severe	− 1.05 (− 0.1–(− 2.3))	2.03	

VEGF, vascular endothelial growth factor

[#] Kruskal–Wallis test;

individual cells, nutrition and differentiation of cells, angiogenesis, and intracellular contact formation [16]. ECM consists of collagenous (different types of collagen) and non-collagenous proteins, such as fibronectin, elastin, laminin, and other components. Elastin, collagen, and proteoglycans are essential factors in the mechanical properties of tissues [17].

Collagen remains possibly the essential component responsible for preserving the structural stability of the tissue. However, the viscoelasticity of biological tissue does not only involve the amount of components' biomechanical properties. It is the product of a dynamic relationship between cells, angiogenic cytokines, and the ECM, such as *VEGF*, which maintains the elasticity and integrity of normal tissues [17]. *VEGF* is a family of closely related growth factors, including several splice variants with numerous biological effects [18]. The production of particular cytokines, including fibroblast growth factor 2 (FGF-2) and *VEGF*, facilitate cell migration and neovascularization in freshly formed scar tissue [19].

Several studies have proved inelastic dartos tissue in hypospadias. The abnormal findings were found in smooth muscle fiber [20], collagen I and VI [4], total collagen, elastin, and reticulin [6]. Even though dartos tissue has abnormal development of some ECM structure, this study found that dartos tissue has well vascularization.

The present study showed a similar expression of *VEGF* in patients with hypospadias compared to the control group. *VEGF* is significantly correlated with the tissue viscoelasticity and the elastic shortening in some organs, such as the cervix. It does not work alone in the elastic mechanism because of crucial tissue mechanical properties such as collagen, elastin, and decorin [9]. A study shows similar blood vessels in the dartos fascia in patients with hypospadias and normal penis [6]. There is strong evidence that this family plays a fundamental role in the growth and differentiation of vascular and lymphatic endothelial cells, but the mechanism remains unclear [9, 18].

VEGF is an angiogenic peptide made from macrophages, endothelial cells, and many other cells [21]. It mediates angiogenesis and facilitates the survival of endothelial cells [10]. Integrin β1 acts as a signal transducer to regulate the mechanical environment during this process. Integrin consists of two subunits of transmembrane that modify conformational structures in response to extracellular force [22]. Previous studies identified integrin as a major mechanoreceptor for extracellular signal sensing [23]. Mechanical stimulation enables integrin subunits to be nucleated and clustered to support the maturation of focal adhesions, such as FAK phosphorylation [24].

Pressure overload, particularly integrin β1, can upregulate and activate integrins [25–27]. Interestingly, FAK activation was dispensable in angiogenic response mediated by the elastic modulus [9]. Instead, an elevated elastic module upregulated integrin β1 expression in H9c2 cells and activated its Talin-dependent downstream targets, Akt and PI3K. Activating PI3K/Akt signaling resulted in *VEGF* being upregulated by its primary transcriptional regulator, HIF-1α [28]. Hypoxia-inducible factor-1 (HIF-1) is a primary transcriptional mediator of the O2 homeostasis hypoxic response. Its master regulator consists of two separate subunits, α, and β [29]. HIF-1 (hypoxia-inducible factor-1) is a key transcriptional mediator of the response to hypoxic conditions [30]. Hypoxia is the primary stimulus for VEGF release [31]. Hypoxia also reduces the power of the tissue and predisposes it for rupture [9]. Increased *VEGF* and paracrine are affected by the elevated ECM stiffness in cardiomyocytes. It subsequently stimulates cardiac angiogenesis and the development of *VEGF*, which can be modulated during cardiac hypertrophy through the elastic modulus of the ECM [8]. Accordingly, Wingate also reported that mesenchymal stem cells in soft *VEGF* matrices display more mature endothelial cell markers than MSC on soft non-*VEGF* matrices [32].

However, this study has several limitations, including small sample size and unequal control allocation, a heterogeneous study cohort with both proximal and distal hypospadias. In addition, the tissue studied is the periurethral dartos collected during the excision of the chordee. Most of the distal hypospadias do not require chordee correction or excision. Moreover, VEGF expression needs to be checked in other tissues before a definite conclusion is drawn.

Conclusions

VEGF expressions might not affect the severity of hypospadias and chordee, implying the pathogenesis is complex involving many growth factors. Further study with a larger sample size is necessary to clarify and confirm our findings.

Acknowledgements
We thank all staffs and nurses who have taken care of patients in our Hospital.

Authors' contributions
PY collected the patient data, and PY, G, and ID analyzed and interpreted data. PY, RP, and FP performed qPCR analysis. PY, G, and ID were major contributors in writing the manuscript. All authors' read and agreed to the final manuscript.

Funding
This study was funded by the Universitas Gadjah Mada (RTA 2021).

Availability of data and materials
All data generated or analyzed during this study are included in this published article. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The Ethical Committee of Faculty of Medicine, Universitas Gadjah Mada/Dr. Sardjito Hospital approved this study (KE/0287/03/2020). Written informed consent was obtained from all parents for participating in this study. All methods were carried out following relevant guidelines and regulations.

Consent for publication
Not applicable.

Competing interests
The authors declared no potential conflicts of interest concerning this article's research, authorship, and/or publication.

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Received: 12 August 2021 Accepted: 18 November 2021
Published online: 27 November 2021

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