

Comparison of Connective Tissue Growth Factor Expression in Urethral Stricture Patients due to Infection and Trauma with IHC and ELISA Methods

Safendra Siregar¹, Nanda Daniswara^{1*}, Jupiter Sibarani¹, Tjahjodjati T¹, Kuncoro Adi¹, Hernowo Bethy², Anglita Yantisetiasti², Ris Kristiana² and Anna Tjandrawati³

¹Department of Urology, Hasan Sadikin General Hospital, Faculty of Medicine Padjadjaran University, Bandung, Indonesia

²Department of Anatomical Pathology, Hasan Sadikin General Hospital, Faculty of Medicine Padjadjaran University, Bandung, Indonesia

³Department of Clinical Pathology, Hasan Sadikin General Hospital, Faculty of Medicine Padjadjaran University, Bandung, Indonesia

*Corresponding author: Nanda Daniswara, Department of Urology, Hasan Sadikin General Hospital, Faculty of Medicine Padjadjaran University, Bandung, Indonesia, Tel: +62813-2577-2888; Fax: +62-291-597805; E-mail: Uronanda@google.com

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Abstract

Objectives: We look for the difference of CTGF expression in urethral stricture due to infection and trauma with Immunohistochemistry (IHC) and Enzyme-linked Immunosorbent Assay (ELISA) methods.

Methods: We measure the CTGF level from 5 urethral stricture tissue of patient with infection, 18 patients with traumatic urethral stricture. We used IHC and ELISA as method of CTGF measurement. We also compared the result between IHC and ELISA in our subjects. Statistical analysis was done using SPSS version 22.0.

Results: From ELISA measurement, the CTGF level of traumatic urethral stricture patients was significantly higher than patients with infection (5.294 ± 7.213 pg/dl vs. 2.62 ± 2.156 pg/dl, $p=0.002$). From IHC examination CTGF expression in trauma group was significantly stronger than infection group ($p=0.041$). There was no significant difference between the result of ELISA examination and IHC examination in measuring CTGF level in urethral infection stricture tissue ($p=1.000$) and trauma stricture tissue ($p=0.112$).

Conclusions: CTGF expression is higher in traumatic urethral stricture patients compared to urethral stricture patients due to infection. There were no significant difference between the result of both ELISA and IHC.

Keywords: Urethral stricture; CTGF; IHC; ELISA; CTGF expression

Introduction

Urethral stricture is an urological disorders with high morbidity rate and average rate of recurrence, and can be increased by the presence of trauma, surgical procedure or infection [1-4]. The pathophysiology of urethral stricture is closely associated with the formation of scar tissue, including the production of the collagen from connective tissue [5,6].

Inflammatory strictures associated with gonorrhea are much less common now than in the past [7]. Strictures treated with urethroplasty today iatrogenic causes account for about half of the urethral stricture cases in the developed world. In about 1 of 3 cases no obvious cause could be identified. The etiology is significantly different in younger vs. older patients and among stricture sites [8]. In Georg Wick review, he postulate that fibrosis is always a sequela of inflammatory processes and that the many different causes of fibrosis all channel into the same final stereotypical pathways [9].

Transforming growth factor beta 1 (TGF-beta 1) upregulation has been implicated in hypertrophic scars and keloids, but it is unclear if it is the cause or an effect of excessive scar formation [10-12]. Little is known about the molecular environment in urethral stricture and the majority of the studies available focused on collagen analysis [13]. Previous studies have shown that the Connective tissue Growth Factor (CTGF) has an important role in the process of fibrosis in various

tissues and organs [1,5]. Therefore, in this study the researchers wanted to see and assess the differences in expression of CTGF in the urethra in relation to the process of fibrosis in patients with urethral strictures due to infection and trauma.

Methods

This study includes preliminary research in a lab where substantially including basic research in patients with urethral stricture in Hasan Sadikin Hospital, Bandung. The design study will be conducted, including experimental research to assess differences in the expression of CTGF in patients with urethral stricture disease due to infections and trauma with immunohistochemistry (IHC) and ELISA parameter.

Inclusion criteria

Patients with urethral strictures who underwent urethroplasty in cases of urethral strictures due to trauma.

Exclusion criteria

Patients with urethral strictures due to infection and trauma who did not undergo urethroplasty.

Examination of IHC and ELISA CTGF expression

IHC: Immunohistochemical examination conducted by using CTGF quantitative technique with 100 times dilution, ultra vision systems/HRP with three-step anti-polyvalent detection system, and conjugated with secondary antibodies. CTGF expression assessed by observing its intensity through IHC staining.

ELISA: ELISA done using semi-quantitative technique by measuring the absorbance value of the bond between antigens with antibodies obtained from ELISA kit [14]. The expression of CTGF obtained by measuring the absorbance value of the bond between antigens with antibodies obtained from ELISA kit [14].

The design of data analysis

All data from the study sample recorded on the form of research to be analyzed. IHC interpretation criteria obtained by an Anatomical Pathology are a categorical variable in accordance with the intensity of staining were assessed.

While the criteria for the interpretation of ELISA obtained from Clinical Pathology expert is a numerical variable which then converted into a categorical using Optical Density detection range CTGF in accordance with ELISA kit which is 0 to 7.5 weak, moderate 7.5-15, 15-30 strong [14].

The data's that have been obtained were tabulated and statistically processed using SPSS version 20.0. The results of this study will be analyzed in an unpaired comparative analysis statistically.

Results

Normality test of the data used to determine the type of statistics used whether parametric or non-parametric statistics. Normality testing using Shapiro Wilk for $N < 50.15$ Data are normal if the Shapiro Wilk test produces a value of significance (P-value) exceeds 0.05.

If the data are normally distributed, then a comparative analysis is done using independent sample t-test while if the data are not normally distributed, then the analysis used was Mann Whitney.

Based on the above Table 1 it can be seen that the normality test results using Shapiro Wilk for both variables of each infection and trauma group using IHC method. Normality test showed that the data distribution is not normal.

	Group	Kolmogorov-Smirnov			Saphiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
IHC	Trauma	0.245	18	0.006	0.802	18	0.002
	Infection	0.300	5	0.161	0.161	0.8835	0.325

Table 1: Normality Analysis Data on IHC Group.

While the normality test results by ELISA as a whole has a significance value above 0.05. This indicates that the data distribution is normal (Table 2).

Analysis univariat result

Acquisition of quantitative data will be presented as the variables involved in the research. Quantitative data is obtained based on the variables and the measurement scale which was stated before.

	Kolmogorov-Smirnov			Saphiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Infection CTGF expression	0.330	5	0.080	0.791	5	0.039
Trauma CTGF expression	0.236	5	0.200	0.916	5	0.002

Table 2: Normality Analysis Data on ELISA Group.

The data that has been provided will be presented in the form of statistical descriptive table in order to facilitate the study results explanation. Here is the data of the variables in the study with descriptive approach statistical tables relating to "Difference between the increase in expression of CTGF in patients with urethral strictures due to infection and trauma" with the following results.

H_0 : There was no significant difference between the increased expression of CTGF in patients with urethral strictures due to infection and trauma.

H_a : There are significant differences between the increased expression of CTGF in normal patients with urethral strictures due to infection and trauma.

$\alpha=0.05$

Criteria:

Reject H_0 if $p\text{-value} < 0.05$

Reject H_a if $p\text{-value} > 0.05$

Based on Table 3, it can be concluded that there are significant differences between the groups of urethral stricture due to infection and urethral stricture due to trauma, and the intensity expression of CTGF IHC in trauma groups stronger than infection group ($p=0.041$).

		IHC				Total	P-value
		Negative	Weak	Medium	Strong		
Group	Trauma	0	7	8	3	18	0.041
		0%	87.50%	72.70%	75%	78%	
	Infection	0	1	3	1	5	
		0%	12.50%	27.30%	25%	22%	
Total		1	8	11	4	23	

Table 3: Results of Comparative Analysis Expression of TGF Patients Urethral Stricture Using IHC Method.

Based on Table 4, it can be seen that the average value of CTGF in the group of patients with infection is 2,620 while the average value of CTGF in the group of patients with trauma is 5.294, which indicates that the value of the expression of CTGF in the group of trauma patients tend to be higher than the value of the expression of CTGF in infection patient group ($p=0.002$).

It is known that p-value less than 0.05 then H_0 is rejected and H_a is accepted so it can be concluded that there is a significant difference between the values of the expression of CTGF in urethral strictures due to infection than trauma.

Group	Elevating Expression of CTGF		
	Mean	Std. Deviasi	P-Value
Infection	2.62	2.156	0.002
Trauma	5.294	7.213	

Table 4: Results of Comparative Analysis CTGF Value to the Patient Urethral Stricture using ELISA Method.

Compliance results of analysis IHC and ELISA

According to the Table 5, Wilcoxon Signed Ranks Test shows comparison of urethral strictures due to infection with IHC and ELISA methods. There are 5 patients with ELISA infection are lower than IHC infection, one person remain same, and 4 patients have higher ELISA infection than the IHC infection. With significance value of 1.000 ($p > 0.05$), it can be concluded that there is no significant difference between IHC infection with ELISA infection.

		N	Mean Rank	Sum Ranks	of
ELISA infection-IHC infection	Negative ranks	5 ^a	4.5	22.5	
	Positive ranks	4 ^b	5.63	22.5	
	Ties	1 ^c			
	Total	1			
		0			
Patients	Variable	Sig.	Conclusion		
Infection	IHC	1	H ₀ Accepted		
	ELISA				

a: ELISA infection < IHC Infection, b: ELISA infection > IHC Infection, c: ELISA infection = IHC Infection

Table 5: Compliance Analysis Result between Examination IHC and ELISA in Group With Infection.

Based on Table 6, Wilcoxon Signed Ranks Test shows a comparison of ELISA and IHC trauma. There are 9 people whose ELISA trauma patients is lower than IHC trauma, 6 remain same, and 3 patients have ELISA trauma patients higher than IHC trauma.

		N	Mean Rank	Sum Ranks	of
ELISA infection-IHC infection	Negative ranks	9 ^a	6.5	58.5	
	Positive ranks	3 ^b	6.5	19.5	
	Ties	6 ^c			
	Total	1			
		8			
Patients	Variable	Sig.	Conclusion		
Infection	IHC	0	H ₀ Accepted		
	ELISA				

a: ELISA infection < IHC Infection, b: ELISA infection > IHC Infection, c: ELISA infection = IHC Infection

Table 6: Compliance analysis result between examination ihc and elisa in group with trauma.

With significance value of 0.112 ($p > 0.05$), it can be concluded that there is no significant difference between IHC trauma and ELISA trauma.

Discussion

Based on the results it can be concluded that the CTGF expression in urethral strictures tissue due to infection and trauma were detected by IHC and ELISA examination. There are significant differences between increased expression of CTGF in patients with urethral strictures due to infection and trauma in which increased expression of CTGF in patients with urethral trauma is higher than infection. This result is likely because urethral strictures due to trauma patients have also been exposed to infection while waiting for an urethroplasty schedule [15-17].

There is no significant difference between the results of IHC and ELISA in urethral strictures due to trauma to the urethral strictures due to infection which indicates that both examination methods can be used in assessing CTGF in urethral strictures tissue due to infection and trauma. Further research in the process of urethral strictures is needed to avoid the occurrence of recurrent stricture. In addition, the presence of evidence that CTGF receptor expression is obtained, research on fibrosis process inhibitor therapy can be developed, especially in the urethral tissue.

Increased expression of CTGF in patients with urethral stricture as a result from trauma is higher than from infection. IHC and ELISA examination can be used to assess the expression of CTGF in urethral strictures tissue due to infection and trauma.

Conflicts of Interest

None of the contributing authors have any conflict of interest, including specific financial interests or relationships and affiliations relevant to the subject matter or materials discussed in the manuscript.

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