mRNA expression

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Submission date: 21-Mar-2021 07:49AM (UTC-0700)

Submission ID: 1538294213

File name: ene_in_Congenital_Cataract_Caused_by_Rubella_Virus_Infection.pdf (497.05K)

Word count: 2493

Character count: 13308

ISSN-0973-9122 (Print) ● ISSN-0973-9130 (Electronic)
Volume 14 number 4, October-Desember 2020

Indian Journal of Forensic Medicine & Toxicology

Website: www.ijfmt.com

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mRNA Expression of PITX3 gene in Congenital Cataract Caused by Rubella Virus Infection

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Abstract

Objective: To analyze mRNA expression of PITX3 gene in congenital cataract caused by rubella virus infection

Methods: Patients under the age of 18 who were diagnosed with congenital cataracts, were tested for anti rubella antibodies, toxoplasma and cytomegalovirus and then underwent cataract surgery, and lens capsule and lens mass samples were taken. Samples taken will be examined for mutation and expression of the PITX3 mRNA gene.

Results: 15 eye samples were included in the study. there was no relationship with rubella infection with PITX-3 gene mRNA expression in the capsule sample and lens mass ($p > 0.05$). However, in terms of average PITX-3 mRNA gene expression was found to be higher in rubella infection compared to non-rubella infection. Based on the examination site, there was no significant difference in expression of PITX-3 mRNA gene between the lens capsule sample and the lens mass ($p > 0.05$). Although there was no statistically significant difference, in terms of the average, the PITX-3 Gen mRNA expression was found to be higher in the lens capsule compared to the lens mass.

Conclusions: Rubella virus infection was the most common cause in the sample, which indicated the need for prevention of rubella infection in pregnant women to reduce the adverse effects of rubella virus infection. PITX3 gene mRNA expression was higher in the Rubella infection group, indicating the possible role of the PITX3 gene in the pathogenesis of congenital cataracts due to rubella virus infection. The average mRNA expression of the PITX3 gene is higher in the lens capsule compared to the lens mass although it does not significantly indicate the important role of the gene in the process of regulating epithelial cells and lens equatorials.

***Keywords:** Congenital cataract, mRNA expression, PITX3 gene, Rubella virus infection*

Introduction

Congenital cataracts are the most common cause of preventable blindness in children throughout the world. The global prevalence of pediatric cataracts ranges from 0.32 to 22.9 / 10000 children and congenital cataracts range from 0.63 to 9.74 / 1000 children, with an incidence between 1.8 and 3.6 / 10000 per year[1].

Hereditary, metabolic, ocular or systemic abnormalities and trauma are factors that are known to play a role in the pathogenesis of cataracts in children. Among the causes that can be prevented are infections[2]. According to reported the incidence of congenital rubella syndrome in Indonesia with hospital-based surveillance and obtained 6% laboratory-confirmed cases and 21.4% clinically compatible cases from 201

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suspected CRS cases[3,4,5]. Among these cases, 66.7% suffered from congenital cataracts. The administration of rubella vaccine in several developing countries has not been widely implemented, causing a high incidence of CRS, especially in Indonesia[6,7].

The exact molecular mechanism of how the virus causes cataracts is also not clearly known. Previous studies have shown that rubella virus interferes with cellular proliferation pathways, changes cytoskeletal structures and induces mitochondrial changes and gives rise to the hypothesis that apoptosis induced by viruses contributes to the teratogenic effects of Rubella virus[8]. The Research conducted by George S, et all prove that the replication of the rubella virus in fetal endothelial cells causes downregulation of genes needed for ear and eye development. It is still not known with certainty which viral gene products and which cellular transcription factors are responsible for the downregulation of these genes[9]. Based on previous studies it can be suspected that the virus can indirectly cause interference with the genes involved in the process of lens formation and development[10].

Genetically hereditary or sporadic congenital cataracts are also very heterogeneous. This is because different mutations in the same gene can cause the same cataract pattern, while the morphology of cataracts varies greatly, where in several families can show the same mutation in a single gene and can cause different phenotypes[11]. Identification of genes that cause congenital cataracts from several causes, whether hereditary, sporadic or infection can help explain the etiology of some cataracts[12].

Some of the genes involved in cataractogenesis include genes that encode crsyallin (CRYA, CRYB, and CRYG), certain lens connexins (Cx43, Cx46, and Cx50) (22), major intrinsic proteins (MIP) or aquaporine, cytoskeletal structural proteins, paired-such as homeodomain transcription factor 3 (PITX3), avian musculoaponeurotic fibrosarcoma (MAF), and transcription factor 4.

One of the genes involved in the process of cataractogenesis is the transcription factor gene. Mutations in this gene have caused abnormalities in the development of the anterior segment, but only three genes, namely PITX3, MAF and HSF4, cause isolated cataracts7. PITX3 is a very important factor for lens formation[4]. Deletions in the gene cause interference with the formation of the lens. Disturbances in these genes can cause lens cells to not differentiate, decrease cell proliferation and increase apoptosis, which will cause interference with lens development[8].

To the best of researchers, there are no studies that report the expression of the PITX3 mRNA gene in patients with congenital cataracts with causes of rubella infection and rubella non-infection by comparing samples from lens lens capsules and lens mass.

Materials and Methods

Subjects and sample collections

15 congenital cataract patients of less than 18 years old who underwent cataract surgery were enrolled in this study. Ethical approval for this study was obtained from the Human Research Ethic Committees of Hasanuddin University. Samples were derived from the lens capsule and lens mass during cataract surgery.

Patients selected were tested for IgM and IgG antibody for rubella, toxoplasmosis and cytomegalovirus. Patients with positive IgM or IgG antibodies for rubella were included in rubella infection group, whereas patients with negative IgM or IgG antibodies for rubella were included in non-rubella infection group.

All patients underwent cataract extraction surgery under general anaesthesia. During surgery, lens capsule and lens mass were collected to be examined for mRNA expression of PITX3 using real time PCR test.The capsules and lens masses specimens were frozen and stored at -80° C until its subjected for RT-PCR analysis.

RNA's Extraction and cDNA Synthesis

Total RNA was extracted from the capsules and lens masses samples using Qiagen RNA extraction kit according to the manufacturer's instructions. The RNAs were reverse-transcribed using Superscript III (invitro gen) .The integrity of RNA and adequate cDNA synthesis were confirmed using beta actin primers. PCR-reaction were initially heated to 65 ° C for 5 seconds for denaturation

Real time PCR examination

For the estimation of PITX3 gene mRNA expression level, real time PCR was performed on SYBR green dye in cDNA derived from total RNA extracted from the capsules and lens masses speciment. Total PCR volume was 20 µl Prior to the amplification, the master mix was prepared by adding 12.5 µl SYBR green mix (Thermo), 0.5 µl cDNA, 1 µl 5 pmol/□l primers targeted PITX3 gene (F: 5' - GCC ACC CTT AGT CCG TG -3 '; R: 5' - GCA AGC CAG TCA AAT G - 3'). 40 times (95°C for 1 minute, 95°C for 15 seconds, 60°C for 30 seconds, and 72°C for 30 seconds) and ended with final extension at 72°C for 10 minutes. The relative expression of targeted gene was calculated as follows: 'D'Ct = Ct (targeted gene) – Ct (housekeeping gene), R = $2^{-(\text{'D'Ct})}$.

Result

Table 1: The Characteristics of Respondents and Congenital Cataract Causes (n = 15)

Characteristic	n	%	Mean±SD
Age (Year)			4,4±2,9
Type of Cataract			
Membranous	3	20,0	
Nuclear	8	53,3	
Total	4	26,7	
Etiology of Catarac			
Rubella Infection	11	73,4	
Non-Rubella Infection	4	26,6	

The results of the analysis of the characteristics and causes of cataracts showed an average age of respondents 4.4 ± 2.9 years, more than half of respondents experienced membranous cataracts (53.3%), congenital cataracts were mostly caused by rubella infection (73.4%) (Table 1)

Table 2: Results of examination mRNA expression of PITX-3 gene in patients with congenital cataracts

Location	mRNA Expression of PITX3 gene (Mean±SD)
Lens Capsul	0,015±0,034
Lens Mass	0,005±0,009

The Results of examination mRNA expression of PITX-3 gene in patients with congenital cataracts were found in lens capsules on average 0.015 ± 0.034 and on lens mass on average 0.005 ± 0.009.(Table 2).

Table 3: Relationship of rubella infection with mRNA expression of PITX-3 gene
In Congenital Cataract Patients

Caused infection	n	Location Ekspresi mRNA Gen PITX-3			
		Capsul Lens		Mass Lens	
		Mean±SD	p	Mean±SD	p
Infection Rubella	11	0,019±0,039	0,602	0,007±0,010	0,695
Non-Infection Rubella	4	0,003±0,003		0,0001±0,002	

Mann Whitney Test

The Results that about" analysis of the relationship of rubella infection with mRNA expression of PITX-3 gene in Congenital Cataract Patients showed no relationship with rubella infection with expression of PITX-3 gene mRNA in capsule and lens mass (p> 0.05) (Table 3).

Table 4: The Differences in mRNA expression of PITX-3 gene in Congenital Cataract Patients based on examination site (n = 15)

Variable	Location	Result	
		Mean±SD	p
mRNA Expression of PITX-3 Gene	Lens Capsul Lens Mass	0,015±0,034 0,005±0,009	0,178

Uji: Mann Whitney Test

The Results of analysis of differences in PITX-3 gene mRNA expression in Congenital Cataract Patients based on the examination site found that there was no significant difference in mRNA expression of PITX-3 gene between capsule and lens mass (p> 0.05). (Table 4)

Discussion

According to the results, the average mRNA expression of the PITX3 gene in the lens capsule is higher than in the lens mass. This can be explained by the fact that the PITX3 gene has a greater role in controlling the lens epithelial cells and at an advanced fetal stage after the lens is formed, the PITX3 protein is found in epithelial cells and lens equators that will control the cell cycle and differentiation of fiber cells[13.]

PITX3 gene mRNA expression in Congenital Cataract Patients found no relationship between rubella infection and non rubella infection groups both expression in the capsule and lens mass. The expression of the PITX3 mRNA gene in both rubella and non-rubella infection cases indicates the importance of this gene in the lens formation process that occurs from the embryo until after birth and even for life in both groups[14.]

The absence of differences in expression levels is probably due to the involvement of other genes or environmental factors that play a role in the process of cataracts in both groups. Although there were no significant differences, the expression of PITX3 gene mRNA was more in the Rubella infection group, indicating the possible role of the PITX3 gene in the pathogenesis of congenital cataracts due to rubella virus infection. PITX3 gene clearly proves to be very important for normal lens development and differentiation. In aphakia (*ak*) mutant, the developing lens does not develop and differentiate, and the lens cells do not form[10.] In addition, lens proliferation is reduced and apoptosis is increased. Based on these studies it was concluded that the abnormal phenotype of the mutant lens is not just one molecular pathway or a specific interaction and is not solely due to the absence of PITX3 expression so that apoptosis, proliferation and differentiation of lens cells are greatly disrupted

Conclusion

Infection by rubella virus was the most commonly found in this study, which signifies the need for prevention of rubella infection in pregnancy to suppress the adverse effects of rubella virus infection. Although statistically it was insignificantly different, PITX3 gene mRNA expression was higher in the rubella infection group, indicating a possible role of the PITX3 gene in the pathogenesis of congenital cataracts caused by rubella virus infection. Increase on average PITX3 gene mRNA expression in the lens capsule compared to the lens mass indicates the important role of the gene in the process of regulating epithelial cells and lens equatorials.

Source of Funding - Self-funding

Conflict Of Interest- None of the authors has competing interests

Ethical Clearance- This research was approved by the Research Ethics Commission of the Faculty of Medicine, Hasanuddin University Makassar, (No. 267/UN4.6.4.5.31//PP36/2020), and all research subjects give written informed consent.

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