

SEROLOGY AND MOLECULAR IDENTIFICATION OF RUBELLA VIRUS IN PEDIATRIC CATARACT AT MOEHAMMAD HOESIN PALEMBANG

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ABSTRACT

Congenital cataract can be found 0.6 - 6 in every 10.000 births. The most etiology is the genetic abnormality but infection is the etiology that can be controlled. Rubella infection is about 10-15 % from all etiology of congenital cataract. Rubella virus is linked with congenital rubella syndrome (CRS). Today there is no data in Indonesia about identification of rubella virus in cataract lens. The purposed of this study to identify rubella virus in children with cataract by blood serology examination and molecular (PCR from lens). The design of this study was descriptive study and this is a pilot study about rubella virus in cataract lens in Indonesia. We had 9 samples - 15 eyes with congenital cataract and juvenile cataract from July 2016 until September 2016. We did blood serological examination IgG rubella, and PCR examination from lens which was taken from aspiration intraoperative. Result: from 15 eyes; 6 eyes PCR (+) with IgG rubella (+) (40 %), 2 eyes (13.33 %) PCR (-) but IgG (+), 4 eyes (26.67 %) with PCR (+) with igG (-), and 3 eyes (20 %) d PCR (-) with IgG (-). Overall from 9 samples there are 7 samples with PCR (+) rubella and 2 samples with PCR (-). There were 7 cases with rubella positive from 9 samples in the lens from pediatric cataract patient during July 2016 until September 2016. PCR examination from cataract lens is much more sensitive than serological blood examination.

Keywords: Congenital Rubella Syndrome, Pediatric Cataract, Rubella Virus, PCR

1. INTRODUCTION

Congenital cataract is a clouding of the lens that occurs since birth which can be caused by various etiological factors that cause visual impairment or visual impairment and blindness in children. The prevalence of congenital cataracts is 0.6 - 6 out of 10,000 live births, but it is estimated that the data in the field globally greater than reported, of which 15% suffer total blindness.^{1,2} The etiology behind the occurrence of congenital cataracts is very much, including can occur due to genetic defects, metabolic disorders,

and infections - both during intrauterine or at birth. Although congenital cataracts related to gene mutation disorders associated with congenital syndrome are the most, but the factor of infection is very important to note, because it is an etiological factor that can be controlled. One of the most important infections that can cause congenital cataract abnormalities is rubella virus infection.¹⁻³

Rubella Virus (RV) was first discovered in the mid-18th century by German scientists, who observed a series of clinical symptoms on the skin resembling eczema. They call it in German as "Roetheln" and until now this

disease is frequent known as German measles. In 1941 an Australian ophthalmologist, Sir Norman McAlister Gregg discovered that there was a link between primary maternal infection and congenital defects that occur in infants with mothers infected with the Rubella virus. Gregg found a strong link between the incidence of congenital cataracts and mothers who were proven to be infected with rubella virus positively. This makes a lot of other research develop so that it is now known that rubella virus infection in pregnant women will cause many effects of congenital defects in various organs. If the infection occurs in the first 12 weeks of pregnancy, it will cause severe fetal damage, even further rubella infection in pregnant women can result in fetal death or abortion. Rubella virus infection in the fetus that is obtained during pregnancy and results in congenital defects is referred to as Congenital Rubella Syndrome or CRS. CRS infections include organs of the eyes, ears, heart, mental retardation, and can even lead to death.²⁻⁴

According to WHO data currently there are 236,000 CRS cases annually in developing countries, where the incidence is around 0.1 - 0.2 of every 1000 live births. In 2014, Grant's study found 141 cases of CRS in 114 countries dominated by Southeast Asian countries. At present CRS numbers are still an iceberg phenomenon, especially in developing countries because not all data can be found, and still low attention to this case. Specifically, in Southeast Asian countries, in 1999 CRS cases were reported to WHO, namely, Indonesia 7 cases, Philippines 2 cases, Thailand 2 cases, and Veitnam 2 cases. But with the active surveillance of CRS cases conducted by the government at this time, with the support of WHO, Indonesia has been able to record a higher number. Among them, 181 cases in Hasan Sadikin Hospital in Bandung during 2008 - 2014, 461 cases during 2008 - 2013 in

Sardjito Hospital Yogyakarta, and 93 cases in Sutomo Hospital in Surabaya during 1993 - 2013. While in our hospital M. Hoesin Hospital Palembang. Until now CRS surveillance data has not been collected, but specifically for congenital cataract cases related to CRS there were 8 cases recorded in the span of 2014-2015. In developed countries CRS cases have declined very far due to the successful "Rubella vaccination strategy" program.¹⁻⁵

Congenital cataracts in infants with mothers who are positive for rubella virus are important points in the overall CRS case assessment. Because mothers who have rubella infection in the first 1-10 weeks of pregnancy will give birth to 90% of infants with CRS and 50% can suffer from cataracts or congenital glaucoma related to rubella.⁴ Where previous studies conducted by G. Shyamala in India have proven that the rubella virus was found living in the lens of infants with congenital cataracts, although blood serology results still show negative rubella IgM.⁶

Meanwhile research is familiar rubella virus in congenital cataract itself is still very little. In Indonesia, there are no studies that specialize in examining the rubella virus in patients with congenital cataracts or rubella cataracts with CRS cases. It is hoped that this research - as a pilot study, can contribute important data for the Indonesian government, especially in M. Hoesin Hospital Palembang. Currently the Indonesian government, especially the Ministry of Health of the Republic of Indonesia is running a CRS surveillance program in several Type A hospitals in various provinces and is also reaching areas where rubella has been reported. This is related to the plan to integrate rubella immunization in the immunization program which will be planned to be included in the routine immunization program in 2017. This program is related to the current WHO global

program for eradication of rubella worldwide, especially developing countries due to the phenomenon of the iceberg detected from due to rubella infection that causes CRS.^{4,6} At present data on the rubella virus genotype that are specifically found in patients with rubella cataracts in Indonesia do not yet exist, therefore research is expected to also be a vital supporting data to support the vaccination program plan the government.⁴⁸ This study aims to identify the presence of rubella virus in eye lens specimens from patients with pediatric cataracts, both congenital cataracts and juvenile cataracts. As well as this study aims to find an association between examination of rubella virus through blood serology and molecular examination with PCR.

2. METHODS

This study is a descriptive study to determine the presence of rubella virus infection in patients with congenital and juvenile cataracts in children aged 0-16 years through blood serology and PCR examination to determine the presence of rubella virus that infects. This research will be conducted at Mohammad Hoesin Hospital in Palembang.

The entire time of implementation starts in July 2016 to September 2016. The target population is all patients diagnosed with congenital cataracts or juvenile cataracts aged 0-16 years who undergo cataract surgery. Affordable population is patients diagnosed with congenital cataracts or juvenile cataracts aged 0-16 years at RS. Hoesin Palembang, who performed cataract operations.

The research sample was determined by a time-based method. The subjects of this study were patients with congenital cataracts and juvenile cataracts aged 0-16 years who had undergone ophthalmological examinations that came to the pediatric

ophthalmic RSMH subdivision and performed cataract surgeries, who had signed the agreement and informed consent within July 2016 - September 2016. The inclusion criteria were (a) All patients diagnosed with congenital and juvenile cataracts aged 0-16 years and cataract surgery performed on the eye. (b) Willing to follow the research stated by signing an informed consent.

The exclusion criteria were (a) History of trauma (b) There is a history of postnatal infections. (c) Diagnosed with other syndromes that are not associated with CRS. (d) Diagnosed with other diseases not related to intrauterine virus infection. (d) Has a history of using old drugs, for example long-term steroids.

All data is displayed in the form of data tabulation and statistical analysis is done using a computer program with the SPSS program, with univariate analysis. Will be analyzed the relationship between the number of patients with IgG (+) and (-) Rubella with PCR results from the lens, the association of IgM (+) or (-) Rubella with PCR results of rubella virus from the lens, and will be analyzed the relationship with a history of infection, especially fever in mother during pregnancy in the first trimester.

3. RESULT

Characteristics of Research Subjects Frequency distribution was based on general characteristics of research subjects which include age, sex, type of cataracts, maternal history and skin rash (Table 1).

Table 1. General Characteristics of Research Subjects

Variable	Frequency (n)	Percent (%)
Age		
≤ 36 months	3	33.33
>36 months	6	66.7
Sex		
Male	5	55.6
Female	4	44.4
Type of cataract		
Juvenile	1	11.1
Congenital	8	88.9
History of Mother Fever		
Positive	9	100
Negative	0	
Maternal Skin Rash		
Positive	1	11.1
Negative	8	88.9

Frequency Distribution Based on examination of Ig. G Rubella and PCR

Table 2. Frequency Distribution Based on examination of Ig. G Rubella and PCR

Variable	Frequency (n)	Percent (%)
Ig.G Rubella		
Positive	5	55.6
Negative	4	44.4
PCR		
Positive	10	66.7
Negative	5	33.3

Frequency distribution based on PCR includes examining the right and left eye in binocular and monocular subjects

Table 3. Frequency distribution based on PCR includes examining the right and left eye in binocular and monocular subjects

Eye	n	PCR Rubella		
		Positive	Negative	Total
Binocular	6	8 (66.7%)	4 (33.3%)	12
Monocular	3	2 (66.7%)	1 (33.3%)	3
	Operated Eye	PCR Rubella		
		Positive	Negative	Total
Binocular n = 6	Right	4 (66.7%)	2 (33.3%)	6
	Left	4 (66.7%)	2 (33.3%)	6
Monocular n = 3	Right	2(100%)	0	2
	Left	0	1 (100%)	1

Rubella virus PCR examination results (Fragment 1)

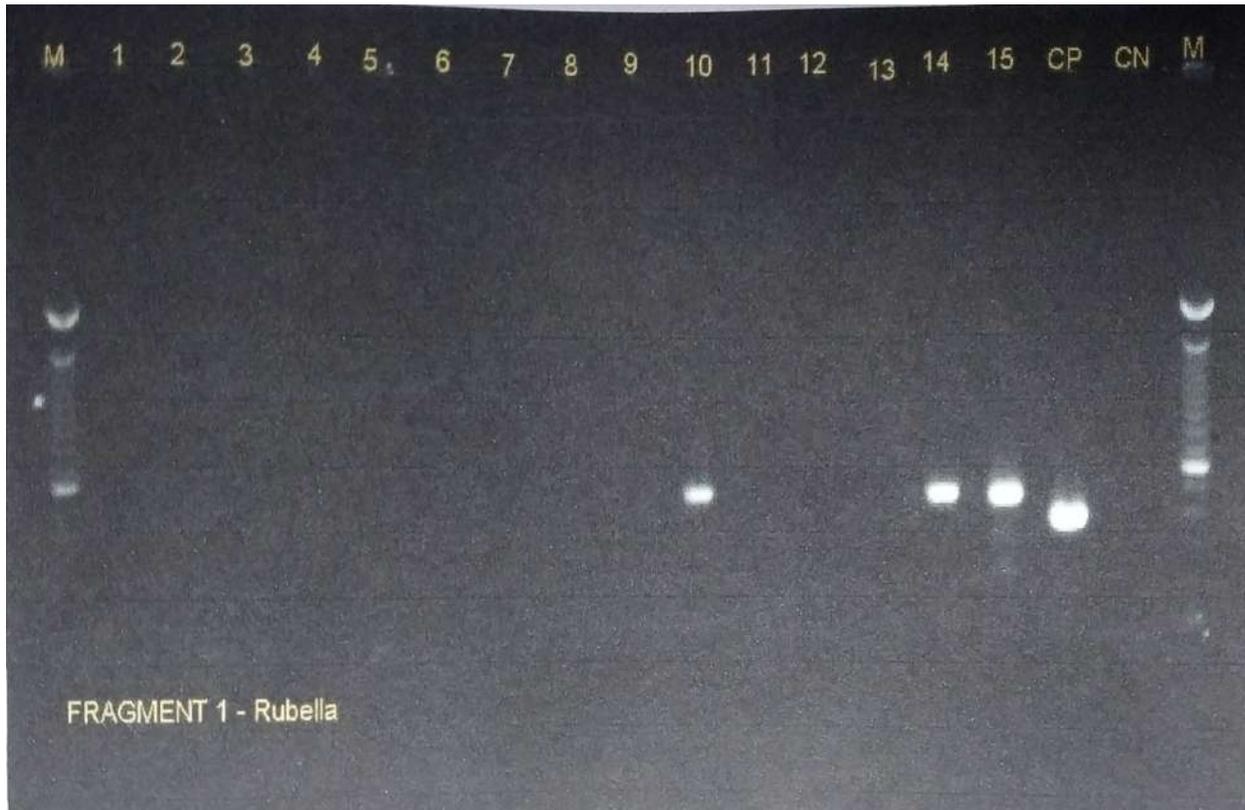


Figure 1. Rubella virus PCR examination results (Fragment 1)

Interpretation of Fragment 1

Number 1	Sample 1 (Left)	Negative
Number 2	Sample 1 (Right)	Positive
Number 3	Sample 2 (Left)	Negative
Number 4	Sample 2 (Right)	Negative
Number 5	Sample 3 (Left)	Positive
Number 6	Sample 3 (Right)	Positive
Number 7	Sample 4 (Left)	Negative
Number 8	Sample 5 (Left)	Positive
Number 9	Sample 5 (Right)	Positive
Number 10	Sample 6 (Right)	Positive
Number 11	Sample 7 (Left)	Positive
Number 12	Sample 7 (Right)	Negative
Number 13	Sample 8 (Right)	Positive
Number 14	Sample 9 (Left)	Positive
Number 15	Sample 9 (Right)	Positive
CP	Control Positive	Valid
CN	Control Negative	Valid
M	Marker (DNA ruler)	

Rubella virus PCR examination results (Fragment 2)

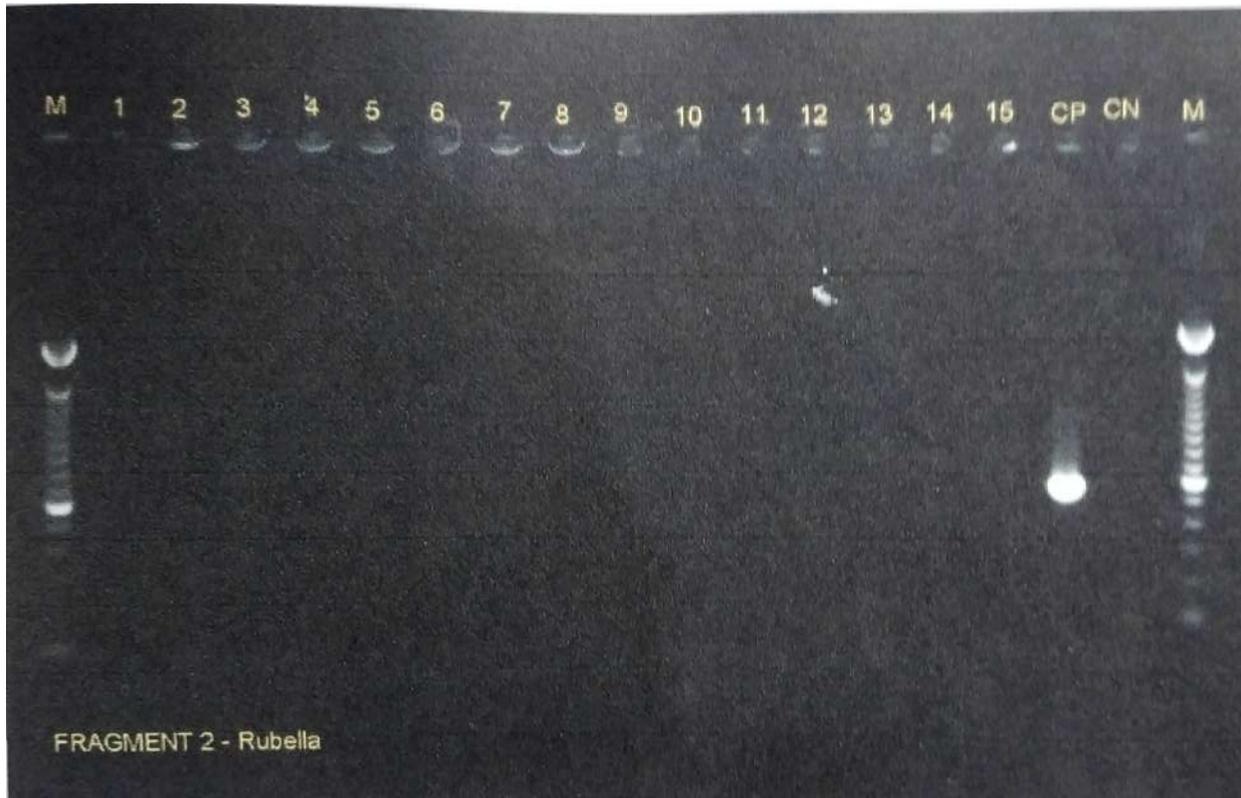


Figure 1. Rubella virus PCR examination results (Fragment 2)

Interpretation of Fragment 2

Number 1	Sample 1 (Left)	Negative
Number 2	Sample 1 (Right)	Negative
Number 3	Sample 2 (Left)	Negative
Number 4	Sample 2 (Right)	Negative
Number 5	Sample 3 (Left)	Negative
Number 6	Sample 3 (Right)	Negative
Number 7	Sample 4 (Left)	Negative
Number 8	Sample 5 (Left)	Negative
Number 9	Sample 5 (Right)	Negative
Number 10	Sample 6 (Right)	Negative
Number 11	Sample 7 (Left)	Negative
Number 12	Sample 7 (Right)	Negative
Number 13	Sample 8 (Right)	Negative
Number 14	Sample 9 (Left)	Negative
Number 15	Sample 9 (Right)	Negative
CP	Control Positive	Valid
CN	Control Negative	Valid
M	Marker (DNA ruler)	

4. DISCUSSION

The subjects in this study group were on average 48 months or 4 years old. With the smallest age is 3 months and the oldest age is 9 years. This relates to the inclusion criteria in this study that all cataract sufferers have congenital cataracts and juvenile cataracts with an age limit of 0-16 years, which are based on the criteria of pediatric patients in Palembang's RSMH where the study is conducted are individuals who are less than 17 years old and have not menstruated. In fact, the selection of the age range in this study is indeed quite large, considering that from other studies research to identify the rubella virus is usually taken by patients aged less than or equal to 3 years. This is consistent with the first study that found that the rubella virus can survive in the lens of the eye until the age of 3 years, namely a study conducted by Menser et al in 1967, and now some literature also reveals the same thing, even considered to be more sensitive if the study was conducted in infants or under 1 year of age. This is in line with the study of Sajani K.Shah in India whose research samples averaged 3.97 months, and G. Shyamala et al in 2008 - in India, which used research subjects less than 1 year old. This is also related to the longer age of the child found rubella virus in the body, the more ambiguous whether the virus infection was obtained maternal or new infection.^{6,7,8,9}

However, in this study conducted at RSMH the research subjects were expanded by taking a wider age coverage due to the number of samples and time constraints. Meanwhile, based on gender, there were more samples of male sex, namely 5 people (55.6%) and those of female sex were 4 people (44.4%). However, the sex of the baby born to mothers infected with rubella virus does not have the meaning of the rubella virus infection that occurs. It also has nothing to do with the incidence of congenital

cataracts. Recognizing the absence of this gender effect is in accordance with much of the reference literature including Nathaniel Lambert et al. From the Mayo Clinic in the United States in 2015, Walter Orenstein in the United States in 2014, and also stated in the main pediatric ophthalmology reference, the American Academy of Ophthalmology 2014-2015. The most types of cataracts performed by cataract extraction patients were congenital cataracts with 8 people (88.9%) while only 1 person was diagnosed as juvenile cataracts (11.1%). What congenital cataracts say is lens opacities that occur at birth and what is said to be juvenile cataracts are lens opacities that occur after passing through infancy.^{10,11}

In this study the type of cataract morphology was not taken into account because the focus of this study was on the presence of rubella virus in the lens, not to find the relationship between the types of cataracts that occur with viral infections. However, the morphology of cataract types still included in clinical data when researchers conducted an ophthalmology examination and written on the attachment to the patient's status. From the history of maternal fever during pregnancy in the first trimester of pregnancy, all mothers of patients (100% 6) claimed to have fever in the first 3 months of pregnancy as research subjects. This research variable has a weakness, because only based on subjective data felt by the mother, can not be assessed the actual temperature, and can not be proven. Mother's skin rash during a fever in the first trimester is also taken into account, because if the patient has a history of rubella infection, then when the body's resistance is low the rash will appear. However, rubella infection in adults can also be without skin rashes.^{2,3,12,5}

In this study found only 1 mother (11.1%) who claimed there was a history of skin rashes in the form of red spots on the skin

during fever in the first trimester and the patient gave birth to a child with Congenital rubella syndrome or CRS, while 8 mothers of study subjects (88.9%) claimed there was no skin patch during fever in the first trimester of pregnancy. This skin rash variable also has a weakness that is only based on the mother's memory and subjective results.

In this study examination of rubella virus infection was carried out, one of which was through blood serology with ELISA, which was IgG and IgM rubella virus IgM examination. Obtained from 9 study subjects, there were 5 subjects who were found positive or 55.6% and there were 4 subjects whose IgG serology results were negative or 44.4%. Nathaniel Lambert et al in his study stated that serological examination is quite sensitive even though PCR examination is more sensitive. This is also in line with Kadek's study in 2007 at Surabaya Soetomo Hospital. Nevertheless, blood serology examination is easier to do and currently all type A government hospitals can do it themselves, and are borne by BPJS. So that the examination standards to prove the presence of rubella virus infection before, blood serology is still used. Positive IgG results based on reference values prove long-standing infections, and there is already immunity against the rubella virus. Even though the bias is negative IgG results, even though rubella virus can be found by PCR or culture in other organs.^{3,6,7,9}

Frequency Distribution Based on PCR Rubella Lens Examination

In this study lens aspiration surgery was performed from 9 subjects, 15 eyes. Where out of 15 eyes the PCR examination with specimens was eyepiece. In a study of 15 eyes found positive rubella virus PCR of 10 eyes (66.7%) and negative eye test results of 5

eyes (33.3%). This could relate to other etiologies of congenital cataracts of research subjects. Where the most etiology of congenital cataracts in the American Academy of Ophthalmology in 2014-2015 and research conducted by Manoj Kumar et al in 2011, the most etiology is genetic disorders. This study found negative PCR was also in line with Malathi Jamu Lingam et al in 2005 in India and G. Shymala et al in 2008 that other viruses that could cause congenital cataracts were CMV, Toxoplasma, and Herpes simplex Virus especially HSV II.

Rubella positive PCR examination in the eyepiece is in accordance with the theory that the rubella virus can survive in the eyepiece of children born from rubella infected during pregnancy. Among them were T. Malala Rajasundari's research in 2013, G. Shymala et al in 2008, Perumalsamy Vijayalakshmi et al in 2001, and Sajani K. Shah in 2014. M. Karkinen Jaaskelainen et al in research at the University of Helsinki in Finland in 1975 were pioneers that proved the existence of rubella virus that lives or dies in the lens of the eye infected with rubella virus, M. Karkinen et al examined 500 embryos from abortion due to therapeutic reasons at the local hospital, and found that rubella virus can stay in the lens of a placode which means that infection occurs during early gestation, some occur when closed stage vesicles - which means it occurs after further gestation, and something is in the lens capsule, not yet entered into the lens core. All occur in infected mothers in the first trimester of pregnancy. In this study on subjects who tested positive for rubella PCR in the eyepiece, 3 subjects were CRS sufferers.

Frequency Distribution of PCR Eye Checks for Binocular and Monocular Patients

In this study it was found that there were 6 patients who were operated on two eyes or binoculars, and there were 3 patients who had only one eye operation - either the right or left eye only. This is related to the patient having had surgery before, or the opacification of the lens in the eye next to it is not too heavy or not yet present. Rubella virus infection during pregnancy is systemic so that it affects both eyes, only the onset differs depending on the virulence and condition of the individual lens.^{1,4,13,14,15}

Frequency distribution based on right-eye PCR obtained by 6 people (75%) positive test results from 8 right eyes examined, while left-eye PCR obtained 4 groups (57.1%) with positive results from 7 eyes examined. This relates to the number of eyes examined, and eyes operated on. There are sufferers who are operated on only the right eye, there are only the left eye, there are both eyes. One operated on because the other eye had not undergone a cataract process, or had done a previous cataract operation. Regarding the right eye or the left eye that is affected has no relevance to rubella infection, rubella infection is systemic, where if one eye is affected then the other eye will also be infected even though the type of abnormality or lens turbidity rate can be different.^{6,7,13,16}

The right eye frequency distribution of binocular subjects was 4 eyes (66.67%) with positive PCR results from 6 right eyes examined, whereas in the left eye 4 eyes (66.67%) had eyes with positive PCR from 6 eyes examined. In monocular subjects, 2 right eyes (100%) were obtained with positive PCR from 2 right eyes examined, and 1 eye (100%) PCR negative in the left eye. Of the 12 eyes operated on in 6 subjects whose binocular operations found positive PCR results in 8 eyes (66.7%), and PCR

negative in 4 eyes (33.3%). While from 3 eyes on monocular operated subjects found positive PCR results in 2 eyes (66.7%) and negative PCR in 1 eye (33.3%). In binocular subjects both eyes must be found positive if one PCR eye is from a positive lens. But on research there are 2 subjects whose eyes were found to be positive PCR while the opposite eye was negative PCR. This is likely due to an error during sampling, or damage to the process of transportation to the laboratory, this needs to be proven by research with larger samples. However, according to Rachel L.Gillespie et al and several other literature confirms that the occurrence of cataracts is bilateral, while in our study subjects there were not positive one side of the PCR, so this might be related to the process of conducting research in the field. In addition to the above differences there is a positive PCR result in one eye while the negative PCR eye is likely also related to the level of turbidity of the lens, or the cataract morphology, as in the study of Bhagabat Nayak et al in India in 2015 which found the most positive PCR in children with cataracts congenital morphology of lamellar cataracts. But in this study conducted at Palembang Hospital RSMH, the morphology of cataracts and the degree of turbidity of the lens, whether complete or not, did not become a research variable and were not taken into account in the study. So that further research is needed with a larger sample for this matter.

Frequency Distribution of PCR Examinations to Ig.G. Rubella Examination

Results of this study show negative PCR results in patients with Ig. G Rubella negative as much as 3 samples (60%) of 5 eyes with negative PCR results, while positive PCR results with Ig.G has positive rubella in 6 samples (60%) of 10 eyes with

positive PCR. So, at the initial examination of the patient coming to the pediatric subdivision eye poles and found a cataract condition on the lens, a blood serology examination will be performed to find any old or new infections. Of 8 patients with rubella positive IgG found positive rubella virus in the eye lens through PCR as many as 6 eyes, and found no rubella virus as much as 2 eyes. This is related to the study of G. Shyamala et al in 2008 in India which found that the most common virus in the lens of a cataracted child was HSV 2. So, there is a possibility that there is another virus that actually causes cataracts in the child despite the positive IgG rubella from the blood. And can also a child have infected with more than one kind of virus such as that also found Malathi Jamu Lingam et al in 2015 which they also found the CMV virus in addition to rubella on PCR lens examination of the eye.

The discovery of rubella virus in the lens of the eye through PCR examination is in line with the findings of Thong Van Nguyen from Vietnam who adopted 3 fetuses that occurred due to abortion in mothers with positive rubella from blood, in Vietnam in 2015, this study was the first to prove that the rubella virus was not only in the lens but also found in the ciliary bodies and lacrimal glands. Hypothesis Thong Van Nguyen et al said that the presence of rubella virus in the epithelial cells of the silier body affects the production of aqueous humor, so that aqueous humor which in this case is responsible for providing nutrition and supply for the lens becomes disrupted so that the resulting turbidity results in the lens. From this theory, it could also be a PCR in the negative lens but the lens remains turbid because the virus damages the silier body so that it remains cataracts occur in the end.

According to pioneering journals such as M.Karkinen et al from Finland in 1975 and Menser et al in 1967 which became a reference for many studies in the world that

the rubella virus can survive in the lens of the eye until the age of children 3 years. But in this study also found positive rubella virus in study subjects aged more than 3 years, namely 3.5 years of age, 6 years two people and 9 years. This finding is in accordance with the study of Winchester et al in 2013 which found the rubella virus living in the 28-year-old Fuch uveltis penedrita in which the sufferer was a baby when diagnosed as congenital rubella syndrome (CRS).

From the results of the study between PCR examinations per research subject and Ig. G examination results rubella found that there were seven samples that had positive PCR from the eyepiece where of the seven study subjects there were 4 samples with positive rubella IgG (57.1%) and three study subjects were IgG rubella negative (42.9%). Samples that have negative PCR results are two samples where one sample is igG rubella positive (50%) and one sample with negative IgG rubella. Chisel from the number of eyes examined, out of 15 eyes there were 6 eyes (40%) of 15 eyes that were positive PCR and blood serum IgG were also positive, there were 2 eyes with negative PCR but positive blood serum results (13.33%), meanwhile, blood serum with IgG rubella was negative but found positive PCR in 4 eyes (26.67%), and the blood serum IgG was negative and similar to PCR from the lens there were also 3 eyes (20%) out of 15 eyes. This study is in line with Walter Orenstein et al in the United States in 2014, T. Malala Rajasundari et al in India in 2013, G. Shyamala et al India 2008, which proved that the most sensitive examination to establish a etiology of congenital cataracts is by PCR examination of the lens. Because the results of negative blood serology can be revealed when a PCR examination on the lens specimen is found to be the true etiology, in this case - rubella virus. This was confirmed by the data of this study, that there were two subjects, with negative blood serology results

from rubella IgG, while high was IgG toxoplasma and another positive subject was IgG CMV, but when lens PCR was examined, it was found that the rubella PCR virus was positive. This finding proves that "silent infection" can occur if the examination is not comprehensive, while if the PCR is positive, further screening should be done whether there are abnormalities related to CRS or not.^{2,3,5,12}

The limited number of very few samples made it difficult to do correlation analysis in this study. This study is also still limited to the RSMH hospital environment so it does not yet represent the characteristics of the population in Indonesia as a whole. The need for more specific research to know the history of mothers during pregnancy because in this study the assessment of new mothers is subjective and has not been assessed clinically and objectively.

5. CONCLUSIONS

Based on the analysis of data obtained in the study, it was concluded that found 7 positive cases of rubella virus through PCR examination on the lens of the eye that developed cataracts in children from 9 cases throughout July 2016 to September 2016 in RS.M. Hoesin Palembang. In this study it was also clear that the PCR examination on the lens was more sensitive than just doing the examination. Rubella IgG and IgM blood serology, due to a negative research subject of rubella blood serology examination results, but apparently rubella virus was found in the cataract's eye lens. Further research with a larger sample size is needed so correlation analysis about this research will provide representative results. Further research also needs to be done from existing samples in the form of sequencing to determine the rubella virus genotype in Indonesia and culture on lenses taken for find whether virus rubella is alive and active or

not. Collective research is needed to be able to study congenital cataracts with other viruses such as CMV, Toxoplasma and HSV. This research can be used as preliminary research to be used as a major program of the Indonesian government through the Ministry of Health of the Republic of Indonesia towards a rubella-free Indonesia and a national rubella vaccine program for women of childbearing age. Research can be a reference to be able to act on the operation of rubella as an active viral infection that is easily infectious through air, and is fatal if it infects pregnant women especially in the first trimester.

REFERENCES

- [1]. Manoj Kumar, Agarwal Tushar, Khokar Shudarshan, Kaur Punit, et al. Mutation Screening and Genotype Phenotype Correlation of g-Crystallin, Y - Crystallin and GJAB Gene in Congenital Cataract. *Molecular Vision*, Vol. 17, 2011: 693-707
- [2]. Lambert Nathaniel, Sterbel Peter, Orenstein Walter, et Rubella. *Mayo Clinic Vaccination Research*. 2015.
- [3]. Orenstein Walter, E Reef Susan. *Rubella Virus. Viral Infections of Human*. Springer Science and Business Media New York, 2014.
- [4]. Nazme Nure Ishrat, Hussain Manzoor, Das Ashith Chandra. *Congenital Rubella Syndrome - A Major Review and Update*. *Delta Med Col Journal* 2015; 3 (2): 89 -95.
- [5]. Kementerian Kesehatan Republik Indonesia. *Pedoman Surveilans Congenital Rubella Syndrome (CRS)*. 2014: 1-5
- [6]. Shyamala G, Sowmya P. Madhavan HN, Maiathi J. Relative Efficiency of Polymerase Chain Reaction and Enzyme Link Immunosorbant Assay in Determination of Viral Etiology in

- Congenital Cataract in Infants. *Journal of Postgraduate Medicine*, Vol 54, No 1, 2008: 17 – 20
- [7]. Jaaskelainen MK et al. Rubella Cataract in vitro: Sensitive Period of the Developing Human Lens. *The Journal of Experimental Medicine*, Vol. 141, 1075: 1238-1248
- [8]. Shah SK et al. Long-term Longitudinal Assessment of Postoperative Outcomes After Congenital Cataract Surgery in Children with Congenital Rubella Syndrome. *J Cataract Refract Surgery* 2014; 40: 2091-2098
- [9]. Menser, MAJ.D.Harley, RHertzberg. D.C Dorman, and AM.Murphy. 1967. Persistence of Virus in Lens for Three Years After Prenatal Rubella. *Lancet* 327
- [10]. Cantor LB, Rapuano CJ, Cioffi GA Lens and Cataract. *American Academy of Ophthalmology Section 11*. 2014-2015. 17-25
- [11]. Cantor LB, Rapuano CJ, Cioffi GA. Childhood Cataracts and Other Pediatric Lens Disorders *American Academy of Ophthalmology Section 6*. 2014-2015: 299-300
- [12]. Kadek, Darmadi S. Gejala Rubella Bawaan (Kongenital) berdasarkan Pemeriksaan Serologis dan RNA Virus. *Indonesian Journal of Clinical Pathology and Medical Laboratory*, Vol. 13. No. 2, Maret 2007: 63-71 75
- [13]. Rajasundari Characterization of Rubella Virus Infection Among Children with Ocular Defects of Congenital Rubella Syndrome. *AECS Illumination*, Vol XIII, No 1, January -March 2013: 1-5
- [14]. Nyuyen Van Thong, Pham Van Hung, Abe Kenji. Pathogenesis of Congenital Rubella Virus Infection in Human Fetuses: Viral Infection in the Ciliary Body Could Play an Important Role in Caractogenesis. *Ebiomedicine*. Elsevier. 2015: 59 - 63 16.
- [15]. Gillespie L Rachel, OSullivan James, Ashwort Jane, Bhaskar Shanjeev, et all Personalized Diagnosis and Management of Congenital Cataract by Next Generation Sequencing. *Journal of American Academy of Ophthalmology*. 2014;121(11):2124-2137
- [16]. Pham Hung Van, Nguyen Thong Van, Nguyen Truc Tanh Thi, Linh Duy Dang, Ngoc, Hieu Hoang, et all, Rubella Epidemic in Vietnam : Characteristic of Rubella Virus Genes from Pregnant Women and Their Fetuses / Newbome with Congenital Rubel Syndrome, *Journal of Clinical Virology Vol 57*, 2013: 152 – 15
- [17]. Dewan Pooja, Piyush Gupta. Burden of Congenital Rubella Syndrome (CRS) in India A systematic Review. *Indian Pediatric*, Vol 49, May 2012: 377 411
- [18]. Manoj Kumar, Agarwal Tushar, Khokar Shudarshan, Kaur Punit, et all. Molecular and Structural Analysis of Genetic Variations in Congenital Cataract. *Molecular Vision*, Vol 19, 2013 : 2436 – 2450.