Immunoexpression Rate of Human Telomerase Reverse Transcriptase in Rapidly Involuting Congenital Hemangioma Patients

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Abstract

Rapidly Involuting Congenital Hemangioma (RICH) is a type of benign tumor that reaches maximum growth at birth and then undergoes involution in the first year after birth. One of the enzymes associated with tumor development and has been acknowledged for its association with the development of several types of cancer is the human Telomerase Reverse Transcriptase (hTERT). This study aimed to analyze the level of hTERT immunoexpression in RICH patients.

The study was conducted on 10 paraffin blocks of RICH patients who were treated at Hasan Sadikin Hospital, Bandung, Indonesia. Samples that were embedded in representative paraffin blocks were stained by using the immunohistochemical staining technique (IHC) with anti-hTERT antibodies. All data were collected and analyzed qualitatively.

The analysis showed that five samples (50%) of RICH patients showed strong hTERT immunoexpression, three samples (30%) of RICH patients showed moderate hTERT immunoexpression, and two samples (20%) of RICH patients showed weak hTERT immunoexpression.

The study concludes that hTERT Immunoexpression detected in RICH patient samples showed very strong of immunoexpression.

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Introduction

Rapidly involuting congenital hemangioma (RICH) is a benign vascular tumor that develops when the baby is in the neonatal period. RICH does not grow at birth, and undergoes involution for 6 to 14 months postpartum.[1-4] Although RICH has specific clinical features, there are still certain difficulties in the diagnostic process given that the clinical features of RICH, non involuting congenital hemangioma (NICH), slowly involuting congenital hemangioma (SICH) and partially involuting congenital hemangioma (PICH) often have similarities. Until now, the diagnosis has generally been based on the speed of involution.[5-12] In addition, Zhang et al. (2020), regarding the involvement of hTERT in the development of liver cancer tumors in humans, it was concluded that hTERT expression was hypothesized to have involvement with tumor development.[13-15] Stopping endogenous catenin expression by catenin gene specific shRNA effectively decreases hTERT expression, suppresses telomerase, and accelerates telomere shortening.[16] This study aims to analyze the level of hTERT immunoexpression in RICH patients.

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Materials and methods

This descriptive research method was conducted retrospectively. The population was taken based on retrospective primary data from the medical records of patients diagnosed with RICH, according to the inclusion and exclusion

criteria in the Anatomical Pathology Department of RSUP Dr. Hasan Sadikin Bandung, from January 2016 to December 2021. Permission to conduct this research was issued by the Padjadjaran University Bandung Indonesia Research Ethics Commission with ethical exemption number 1096UN6.KEP/EC/2022.

The sample size was taken using the rule of thumb with a benchmark for the number of independent variables examined. One rule of thumb is that the number of subjects required is 10 times the number of variables. So if the number of variables is one (1), then between 10 is needed. According to the rule of thumb, this research requires a sample of at least 10 research subjects that match the inclusion and exclusion criteria.[20]

The research sample was taken by purposive sampling according to the sequence of patient data that met the inclusion criteria. Recording of age, sex, clinical description, histopathological diagnosis was taken as secondary data based on medical records. The representative paraffin block embedded sample was then stained using the immunohistochemical staining technique (IHC) with anti-hTERT antibody.

The inclusion criteria in this study were:

1) Data on medical records of patients with a diagnosis of RICH.
2) Paraffin blocks in good condition and in accordance with medical records selected at the Departement Anatomical Pathology of Hasan Sadikin General Hospital.

The exclusion criteria in this study were:

1) Incomplete RICH patient medical record data.
2) The preparation is not clearly legible.

The paraffin block from the preparation that had been selected as the research sample was cut 4 microns thick using a rotary microtome as much as 1 preparation. Then it was placed on a slide to be stained with anti-hTERT antibody (the brand: Antitelomerase reverse transcriptase antibodies, manufacturer: Abcam biotechnologi company-USA).

Immunohistochemical assessment of hTERT antibodies was carried out on tissue preparations that had been made with a diagnosis of RICH in the patient's medical record. The hTERT expression level was assessed by a semi-quantitative score including the intensity of the entire area of the lesion that was visible at the time of staining of the endothelial cells as brown. hTERT expression was analyzed in the lumen of blood vessels.[8,11,16-18]

Then the data was collected and univariate analysis was carried out. This analysis was used to describe the research variables in the form of tables and graphs to provide a description of the characteristics of the respondents, localization of RICH sufferers and hTERT immunohistochemical examination expressed in RICH patient samples, and used SPSS version 26.0.

Results

Research on the level of hTERT immunoexpression in RICH samples was carried out on 10 paraffin block samples at the Anatomical Pathology Department of RSUP Dr. Hasan Sadikin Bandung, in the period January 2016-December 2021. The research sample consisted of 10 samples with RICH diagnoses and the icd code 10 was D18.0, with the following data :

Gender	Age (years)	Location	hTERT Expression Power
Baby Boy	1	Brachi dextra	Weak
	1	Region ala nasi	Strong
	0	Hemithorax sinistra	Strong
	2	Hemithorax sinistra	Moderate
	1	Shoulder region	Strong
	1	Hemangioma nose	Weak
Baby Girl	0	Labia oris	Strong
	2	Labia oris	Moderate
	1	Genu dextra	Moderate
	0	Antebrachii Sinistra	Strong
Total	10		

Table 1. Sample Characteristics, Location of RICH, and Levels of hTERT Immunoexpression in RICH patients.

From the table it can be seen that most of the respondents were male, and the locations where RICH was found more frequently were the left hemithorax and labia oris. There was evidence of hTERT immunoexpression in RICH patient samples. hTERT immunoexpression in RICH patient samples was strongly expressed in 50% of the study samples, moderately expressed in 30% of the study samples, and weakly expressed in 20% of the study samples.

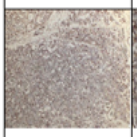
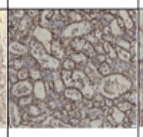
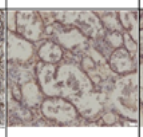
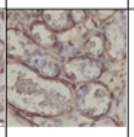
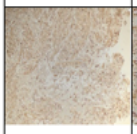
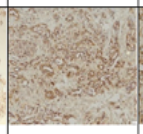
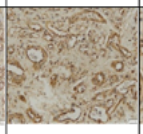
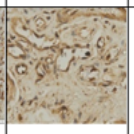
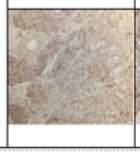
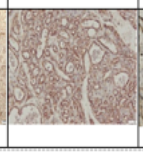
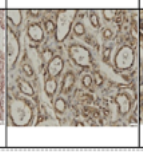
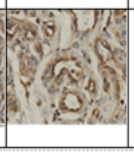
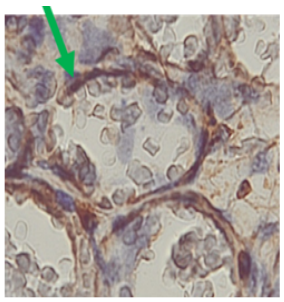
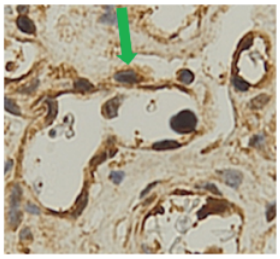
hTERT Expression Photo	20x	100x	200x	400x	hTERT Expression Criteria
					Weak (sample code 154864)
					Moderate (sample code 198018)
					Strong (sample code 191181)

Table 2. Photo documentation results of research on hTERT expression in RICH.



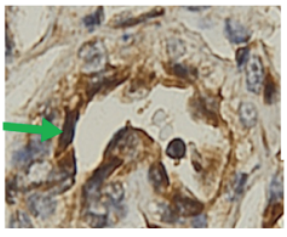
Here's a sample photo at 400x magnification. The arrows show one of the RICH nuclear endothelial cell where hTERT immunoexpression is shown in a weak category. Nuclear endothelial cell show a light brown/light color. The sample preparations were stained brown on the nuclear endothelial cell of blood vessels with an average of more than 80%.

Figure 1. Immunoexpression of hTERT in a sample of RICH patients with a weak category.



Below is a 400x magnification photo. The arrows show one of the RICH nuclear endothelial cell where hTERT immunoexpression is seen in the moderate category. Nuclear endothelial cell show a more magenta brown color. The sample preparations were stained brown on the nuclear endothelial cell of blood vessels with an average of more than 80%.

Figure 2. Immunoexpression of hTERT in moderate category RICH patients.



The following is a sample photo with a 400x magnification. The arrows show one of the RICH Nuclear endothelial cell where hTERT immunoexpression is shown with a strong category. Nuclear endothelial cell show a darker brown/dark brown color. The sample preparations were stained brown on the nuclear endothelial cell of blood vessels with an average of more than 80%.

Figure 3. Immunoexpression of hTERT in RICH patients with strong category.

hTERT immunoexpression in this study was categorized into weak, moderate, and strong categories. Magnification was performed at 20x, 100x, 200x, and 400x using an Olympus BX51 microscope. hTERT immunoexpression will be classified into the weak category if the color is light brown, that is light brown, the color is similar to ocher/clay, but slightly darker; belonging to the moderate category if it shows a brown color image with a slightly higher red or magenta value than "ordinary" brown; and is included in the strongly expressed category if it shows a dark brown/ darker color with normal brown color. The categorization of hTERT immunoexpression in samples of patients with RICH can be observed in Table 1.

Discussion

In this study, six samples were found from rapidly involuting congenital hemangioma RICH patients with male sex and four samples came from RICH patients with female sex. This insignificant difference in the incidence of men and women is in line with the research conducted by Warang-Angin at Palembang General Hospital in 2019, where it was found that the number of female patients was 55.74%, and male patients were 44.26%. Furthermore, in this study it was found that RICH was most commonly found in the left Hemithorax and Labia oris. This is in accordance with the literature where rapidly growing congenital hemangiomas are usually located in the extremities, head and neck region and may be associated with decreased gioplatelet, thought to be due to local intravascular coagulation.[19]

Most of the human telomerase reverse transcriptase (hTERT) immunoexpression of RICH patients was in the strong category (Table 1). In the study of Betty et al. (2014), hTERT expression levels in infantile hemangioma tissue during the proliferative stage were significantly higher than in normal tissue at the degenerative stage and around the tumor, with significant differences. Positive-expressing cells are scattered, resembling hemangioma endothelial cells, and are located in the cytoplasm and nucleus (cytoplasm is brown and nucleus is blue and brown and yellow).[16] This difference in strong, medium and weak expression is related to the growth stage of the RICH (figure 1,2 and 3). According to the literature, hTERT is commonly expressed during the proliferative period. This is in accordance with the literature which states that telomere synthesis is catalyzed

by the telomerase enzyme, which has low activity or is difficult to detect in normal human cells, but is increased in most tumor cells. In addition to the nucleotides that make up telomere deoxyribonucleic acid (DNA) strands, there are also various proteins that together carry out telomere functions.[21]

The pathogenesis of hemangiomas occurs due to disruption of the process of angiogenesis and vasculogenesis which causes uncontrolled proliferation of vascular elements. Tumor angiogenesis influences the process of RICH events.[3-6] In RICH there is an overgrowth of the blood vessels. hTERT expression in vascular tumor lesions and increased expression induced by vascular endothelial growth factor (VEGF) which is a blood vessel stimulator in RICH formation.[11-14]

In this study, hemangiomas were found in the nose and lip area. Depending on the location, hemangioma located in the orofacial area can cause aesthetic and functional disturbances. The most common location for hemangioma to occur is the lips, but hemangiomas can also be found in other areas, such as the tongue, buccal mucosa and palate. Usually occurs soon after birth or early in infancy; although some cases show the time of occurrence in adulthood.[14]

The results of this study are expected to be used as clinical markers in determining the prognosis of RICH patients. Because Powter B, et al (2021) stated that telomerase reverse transcriptase promoter (pTERT) mutations can be used as clinical markers and have potential clinical use in early detection, prognostication, and monitoring of cancer development.[18,19] The detection of hTERT messenger ribonucleic acid (mRNA) levels, as a tumor marker, can reflect the tumor burden and clinical status of the patient.[19,21]

Conclusions

There is evidence of hTERT immunoexpression in RICH patient samples and immunoexpression of hTERT in RICH patient samples is expressed in the very strong. Immunexpression of hTERT is more common in male patients than in female patients and commonly found in the left hemithorax and labia oris.

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Declaration of Interest

The authors report no conflict of interest.

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