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ARAŞTIRMA / RESEARCH

Relationship of telomerase activity with prognosis in patients with neuroblastoma

Nöroblastom hastalarında telomeraz aktivitesinin prognoz ile ilişkisi

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Abstract

Purpose: The aim of this study was to investigate the effect of telomerase activity on clinical characteristics and survival of patients with neuroblastoma.
Materials and Methods: This study was carried out by examining telomerase activity in previously taken pathology preparations of 87 patients who were diagnosed with neuroblastoma between 2011 and 2019 in the Pediatric Oncology Department of Çukurova University Faculty of Medicine.
Results: Totally 87 patients, 46 males (52.9%) and 41 females (47.1%), were included in our study. Median age at the time of diagnosis was 3.0 (0.08-15) years. Twenty-three (26.4%) patients included in the study had telomerase activity, 64 (73.6%) had no telomerase activity. Overall survival (OS) of patients with telomerase activity up to 8 years were 84%, while event-free survival (EFS) was 56%. Patients without telomerase activity had a 93% OS for up to 8 years, while EFS was 70%. There was no significant relationship between patients with or without telomerase activity in terms of sex, age, stage, risk group, relapse, myc-n amplification, OS and EFS.
Conclusions: In this study, no significant difference was found between telomerase activity and clinical features and survival of neuroblastoma patients. Prospective studies involving larger numbers of patients will more clearly demonstrate the impact of telomerase activity on the prognosis of patients with neuroblastoma.
Keywords: Neuroblastoma, telomerase activity, prognosis, children

Öz

Amaç: Bu çalışmanın amacı, telomeraz aktivitesinin nöroblastomlu hastaların klinik özellikleri ve sağkalımı üzerindeki etkisini araştırmaktı.
Gereç ve Yöntem: Bu çalışma, Çukurova Üniversitesi Tıp Fakültesi Pediatrik Onkoloji Anabilim Dalı'nda 2011-2019 yılları arasında nöroblastom tanısı konulan 87 hastanın daha önce alınan patoloji preparatlarında telomeraz aktivitesi incelenerek yapılmıştır.
Bulgular: Çalışmamıza 46'sı erkek (%52.9) ve 41'i kadın (%47.1) olmak üzere toplam 87 hasta dahil edildi. Tanı anındaki ortanca yaş 3.0 (0.08-15) yıldı. Çalışmaya alınan 23 (%26.4) hastada telomeraz aktivitesi mevcutken 64 (%73.6) hastada telomeraz aktivitesi yoktu. 8 yıla kadar telomeraz aktivitesi olan hastaların genel sağkalımı (OS) %84 iken olaysız sağkalım (EFS) %56 idi. Telomeraz aktivitesi olmayan hastalar 8 yıla kadar %93 OS'ye sahipken, EFS %70 idi. Telomeraz aktivitesi olan ve olmayan hastalar arasında cinsiyet, yaş, evre, risk grubu, relaps, mycn amplifikasyonu, OS ve EFS açısından anlamlı bir ilişki yoktu.
Sonuç: Bu çalışmada, telomeraz aktivitesi ile nöroblastom hastalarının klinik özellikleri ve sağkalımları arasında anlamlı bir fark bulunmadı. Daha fazla sayıda hastanın dahil edileceği prospektif çalışmalar telomeraz aktivitesinin nöroblastom hastalarının prognozu üzerine etkisini daha net bir şekilde ortaya koyacaktır.

Anahtar kelimeler: Nöroblastom, telomeraz aktivitesi, prognoz, çocuklar

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INTRODUCTION

Neuroblastoma is the most common extracranial tumor in children and accounts for about 8-10% of childhood cancers. Due to its variable biological structure, the tumor can exhibit quite different clinical and biological behaviors, it is a tumor that can regress spontaneously, as well as it can show rapid progression in patients under one year of age[1-2]. The etiology of neuroblastoma is not exactly known, it is known that the occurance of neuroblastoma is caused by genetic and environmental factors. These factors are thought to be caused by gene amplification, chromosome gain, oncogene activation, allelic losses, tumor suppressor gene losses and alterations in the expression of some genes[3]. As the information about biological and genetic changes increase, which are important indicators of response to treatment and prognosis, advancements in the treatment of the disease also increase.

It is believed that the reactivation of the telomerase enzyme is one of the most important mechanisms for the cells to gain the ability to reproduce indefinitely[4]. It was also proposed that telomerase expression in patients with neurofibromatosis type-1 can be used as a useful marker for tumorigenesis[5]. Telomerase reactivation has been found predominantly in many human malignancies, and this suggests that telomerase activation plays an important role in malignant tumor growth, but in addition to that there is no telomerase activity mainly in normal somatic human tissue[6].

Chromosomal abnormalities involving the human telomerase reverse transcriptase were reported in patients with different cancer types, including glioblastoma, hepatocellular carcinoma, bladder cancer, melanoma and non-melanoma skin cancer, urinary tract cancer, and thyroid cancer[7]. Additionally Telomerase gene therapy has been recognized as one of the key targets to develop efective treatment for cancer[8]. For this reason, the enzyme telomerase has become important in both the diagnosis and treatment of cancer. This study was conducted to investigate the effect of telomerase activity on clinical characteristics and survival of patients with neuroblastoma.

MATERIALS AND METHODS

This study was conducted by examining telomerase activity in the pathology preparates of 87 patients diagnosed with neuroblastoma between 2011-2019 in the Division of Pediatric Oncology of the Faculty of Medicine of Çukurova University. The Ethical Committee of Çukurova University Faculty of Medicine approved the study on 01.06.2018 (meeting number 78). In addition, biopsy materials were obtained from the Department of Pathology of the Faculty of Medicine of the Çukurova University. Tissue samples were extracted by Ş.E. in the Department of Pathology and telomerase activity was studied by Ş.E. and Ç.B. in the same department. The files of the patients who participated in our study were reviewed retrospectively. Date of birth, date of application, age, gender, medical history, risk factors, myc-n amplification, vanillylmandelic acid (VMA) level, treatment modalities, the last status of the patients, relapse, and progression with dates were recorded from the files and the automation system.

1-18 years old patients who were diagnosed with neuroblastoma and ganglioneuroblastoma, with adequate pathologic material sample and who recieved their treatment in our center were included; while patients without adequate pathologic material, and diagnosed in another center were not included. Between the specified dates, 106 patients were diagnosed with neuroblastoma. After the removal of 13 patients who did not have sufficient tissue samples and 6 patients whose treatment was continued in another center, the remaining 87 patients were included in the study.

High pure FFPET RNA isolation kit application steps

Tissue samples which belong to the patients were deparaffinized step by step with the Roche High Pure FFPET RNA Isolation kit. The tissue parts, deparaffinization of which were completed were put in to a sterile 1.5 ml ependorf tube. 100 µl RNA tissue lysis buffer, 16 µl 10% SDS and 40 µl Proteinase K were added. The mixture was vortexed and spinned down. Shaken at 600 rpm and left for 30 minutes at 85°C. At the end of the process, the tube was spinned down and cooled to 55°C. 80 µl of Proteinase K was added. It was shaken at 600 rpm and left for 30 minutes at 55°C. At the end of this stage, lysate should be clearly visible, if there was a particle, processed another 10 minutes. 325 µl RNA binding buffer and 325 µl absolute ethanol were added.

The mixture was vortexed and taken into high pure filter tubes, and an high pure collection tube was placed under it. It was then centrifuged for 30

seconds at 8000 rpm and the collection tube was replaced. It was centrifuged for 2 minutes at 8000 rpm to dry the filter and 100 µl of DNase working solution was added and left at room temperature for 15 minutes.

500 µl wash buffer 1 was added and centrifuged for 20 seconds at 8000 rpm, then the tube was emptied. It was centrifuged at 13200 rpm for 2 minutes to dry up the filter. Then a clean eppendorf tube was placed under the high pure filter tube. 25-50 µl elution buffer was added and left at room temperature for 1 minute. It was centrifuged at 8000 rpm for 1 minute and total RNA was extracted. At the end of these studies, samples with telomerase activity are determined by the device as they exhibit radiation and were recorded as having telomerase activity (between 30-40 cycles), while images that did not form a wavelength and did not exhibit radiation in the device were recorded as having no telomerase activity.

Statistical analysis

IBM SPSS Statistics version 20.0 package program was used for the statistical analysis of the data. Categorical measurements were summarized as numbers and percentages, and numerical measurements were summarized as mean and standard deviation. Chi-square test statistics were used for the comparison of categorical measurements like age group and sex between groups. Log-rank test was performed under Kaplan-Meier analysis to examine the relationship between patient characteristics such as stage, risk group, myc-n amplification, recurrence and overall survival (OS) and event-free survival (EFS). P values <0,05 were considered significant.

RESULTS

A total of 87 patients were enrolled in our study, involving 46 (52.9%) males and 41 (47.1%) females. Median age at the time of diagnosis was 3.0 (0.08-15) years. 32 (36.8%) of our patients were in low stage, 55 (63.2%) were in advanced stage and again, 34 (39.1%) of these patients were in low-risk group, 53 (60.9%) were in high-risk group. Among the patients included in the study; 9 (10.3%) were myc-n gene amplification positive, 45 (51.7%) were negative, 33 (37.9%) have not been studied with myc-n gene amplification, also 31 (37.9%) of the patients had high VMA levels in the urine during diagnosis, 55 (63.2%) were normal, and 1 (1.1%) has not been studied in patients (Table 1).

Table1. Characteristics of patients with neuroblastoma

Variable	n(%)
Stage	
Low	32 (36.8)
High	55 (63.2)
Risk Group	
Low	34 (39.1)
High	53 (60.9)
Myc-n	
Yes	9 (10.3)
No	45 (51.7)
Not studied	33 (37.9)
VMA	
Yes	31 (35.6)
No	55 (63.2)
Not studied	1 (1.1)
Treatment	
Received	67 (77)
Not received	20 (23)
Radiotherapy	
Received	20 (23)
Not received	67 (77)
MIBG	
Recieved	9 (10.3)
Not received	78 (89.7)
BMT	
Done	13 (14.9)
Not done	74 (74.1)
Relapse	
Yes	20 (23)
No	67 (77)
Last Status	
Alive	62 (71.3)
Exitus	17 (19.5)
Lost	8 (9.2)

BMT: Bone marrow transplantation, MIBG: Meta-Iodobenzylguanidine, VMA: Vanillylmandelic acid

In our study, neuroblastoma patients received an average of 91% overall probability of survival for up to 8 years during the 29.6 months (0.66-93.8) follow-up. In the same way, all neuroblastoma patients had a 66% chance of EFS for up to 8 years during 29.6 months of follow-up.

There were a total of 87 patients in our study, and 23 (26.4%) of these patients had telomerase activity, and 64 (73.6%) had no telomerase activity. In terms of the relationship of telomerase activity with sex, the ratio

of those with telomerase activity in girls was 26.8%, while the ratio of those without telomerase activity was 73.2%, and the ratio of men with telomerase activity was 26.1%, and the ratio of those without telomerase activity was 73.9% (p=0.1). No significant association was found between sex and telomerase activity in the patients we enrolled in the study. In patients between 0-18 months, the proportion of those with telomerase activity was 8.7%, while the proportion of those without was 24.3%. In patients older than 18 months, the proportion of those with telomerase activity was 14.3%, while the proportion of those without was 39.7%. Statistically, no significant difference was found (p=0.54).

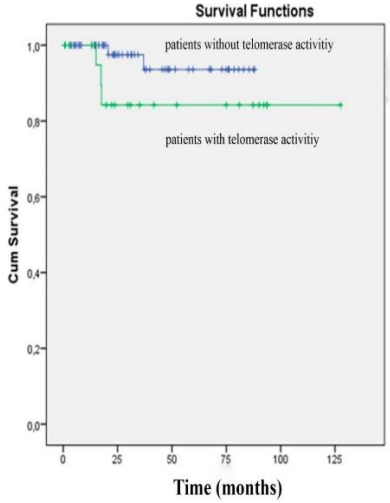

Figure 1. Relationship of telomerase activity with life span (log rank p=0.112).

Patients with telomerase activity had an OS of 84% up to 8 years, while patients without telomerase activity had 93% up to 8 years, and there was not any significant difference in the compared life expectancy (p=0.112) (Figure 1). In our patients enrolled in the study, those with telomerase activity had a 56% probability of EFS for up to 8 years, while those without telomerase activity had a 70% probability of EFS for up to 8 years (p=0.222) (Figure 2).

In the low-stage group, the proportion of patients with telomerase activity was 31.2%, while the proportion of patients without telomerase activity was 68.8%. In the high-stage patient group, the proportion of patients with telomerase activity was 23.6%, while the proportion of patients without telomerase activity was 76.4% (p=0.600). There were no statistically significant differences. In the low-risk group patients, the proportion of patients with telomerase activity was 32.4%, while the proportion of patients without telomerase activity was 67.6%. In high-risk patients, the proportion of patients with telomerase activity was 22.6%, while the proportion of patients without telomerase activity was 77.4% (p=0.451). No significant difference was found between the risk group and telomerase activity.

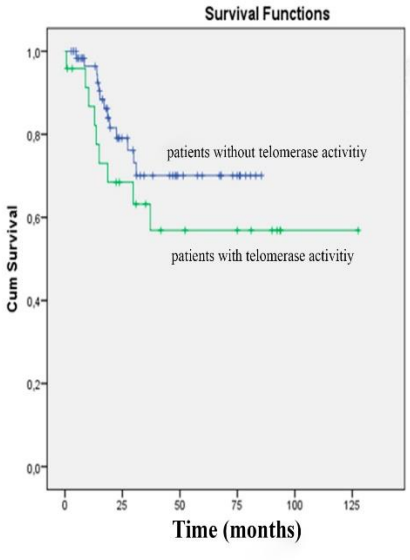

Figure 2. Possibility of event free survival (log rank p=0.222).

33.3% of the patients with positive Myc-n amplification had telomerase activity, while 66.7% had no telomerase activity. 26.7% of the patients without Myc-n activity had telomerase activity, while 73.3% had no telomerase activity (p=0.854). Statistically, there was no significant difference between Myc-n amplification and telomerase activity. 35% of patients with relapses had telomerase activity, while 65% had no telomerase activity. 16% of the patients without relapses had telomerase activity, while 51% had no telomerase activity (p=0.484) (Table 2). The association between relapse and telomerase activity was not statistically significant.

Table 2. Association of neuroblastoma patients with telomerase activity.

Telomerase activity	Positive n (%)	Negative n (%)	*P* value
Stage			
Low	10 (31.2)	22 (68.8)	0.600
High	13 (23.6)	42 (76.4)	
Risk			
Low	11 (32.4)	23 (67.6)	0.451
High	12 (22.6)	41 (77.4)	
Myc-n			
Yes	3 (33.3)	6 (66.7)	0.854
No	12 (26.7)	33 (73.3)	
Relapse			
Yes	7 (35)	13 (65)	0.484
No	16 (23.9)	51 (76.1)	

DISCUSSION

In our study, the overall survival probability of neuroblastoma patients was 91% up to 8 years, and the probability of uneventful survival was 66% up to 8 years. In a study conducted by Piette et al. with 23 neuroblastoma patients, OS up to 5 years was 83%, and EFS up to 5 years was 75%[9]. In another study on neuroblastoma patients, overall 5-year survival rates were confirmed to be 96% in the observation group, 89% in the medium-risk group and 50% in the high-risk group[10].

A total of 87 patients were included in our study, and 23 (26.4%) of these patients had telomerase activity, while 64 (73.6%) had no telomerase activity. In a study by Peifer et al. a complete genome sequencing of 56 neuroblastoma patients (high-risk, n = 39; low-risk, n = 17) was conducted and repetitive genomic rearrangements affecting a chromosomal region in the 5p15.33 proximal telomerase revers transcriptase gene (TERT) was discovered. These rearrangements were only seen in 12 (31%) of high-risk neuroblastoma patients, and in 21% of all neuroblastoma patients[11]. In another study, 105 neuroblastoma patients were examined and 23 (22%) had high telomerase activity, 78 (74%) had low activity, and 4 (4%) had no telomerase activity[12].

In our patients enrolled in the study, those with telomerase activity had a 56% EFS for up to 8 years, while those without telomerase activity had a 70% survival rate for up to 8 years, there was no statistically significant difference (p=0.222). Nozaki et al. examined the prognostic impact of telomerase activity in patients with neuroblastoma, in that study 5-year EFS rate in patients with low telomerase activity was % 86.5, while in patients with high telomerase activity was 53.8%. The 5-year EFS rate viewed with a combination of telomerase activity and TrkA expression was highest among patients with high TrkA expression and low or nonexistent telomerase activity (91.7%), while EFS was lowest in those with low TrkA expression and high telomerase activity. It is therefore thought that telomerase activity will be a useful prognostic factor for neuroblastoma[13].

In this study, since we are a reference center and the patients arrived at an advanced stage because of the long distances, some of our patients interrupt their treatment and continue treatment in other centers, likewise sometimes because of bed shortage we refer patients to other centers, all of which led to a large number of non-follow-up patients. Because it is a retrospective study, pathology specimen of some patients could not be attained (patients diagnosed in other facilities were also present), or unable to work on due to inadequate sampling and the lack of pathology specimen prevented myc-n amplification, a significant prognostic indicator. In addition, after the civil war that began in Syria, many refugee patients were treated in our institutions. As we pointed out in another report some patients have arrived with advanced disease at the time of diagnosis or during the treatment due to delays in diagnosis and treatment because of conflict and war, leading to poor outcomes[14]. Reasons such as the lack of regular follow-up of this group of patients because of barriers in language, poor hygiene conditions, difficulties in supply of medicines and blood products, lack of accommodation created limitations in our study.

As a result, in this study, we did not find a significant association between telomerase activity and the

variables we examined, but we found that survival rates were better in group of telomerase negative patients. A wide range of prospectively designed series are needed to better evaluate the relationship of telomerase activity with risk groups and survival in neuroblastoma patients. Prospective studies involving larger numbers of patients will more clearly demonstrate the impact of telomerase activity on the prognosis of patients with neuroblastoma.

Yazar Katkıları: Çalışma konsepti/Tasarımı: SK, ŞE, ÇB; Veri toplama: ÇB; Veri analizi ve yorumlama: SK; Yazı taslağı: ÇB, SK; İçeriğin eleştirel incelenmesi: SK, GS, İB; Son onay ve sorumluluk: SK, ÇB, ŞE, GS, İB; Teknik ve malzeme desteği: SK, ÇB, ŞE; Süpervizyon: SK, ŞE, GS, İB; Fon sağlama (mevcut ise): yok.
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