Yeni Üroloji Dergisi - *The New Journal of Urology* **2023;18(2):115-123. doi: 10.33719/yud.2023;18-2-1150164**

Original Research / Özgün Araştırma

Investigation of Cyclin-D1 immunohistochemical expression in bladder urethelial carcinoma

Mesane ürotelyal karsinomlarında Cyclin-D1 immünohistokimyasal ekspresyonunun araştırılması

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Geliş tarihi (Submitted): *2022-07-28*
Kabul tarihi (Accepted): *2023-03-30*

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Özet

Amaç: Cyclin D1 hücre siklusunun düzenlenmesinde görev alan ve CCND1 geni tarafından kodlanan bir proteindir. Liteatürde Cyclin D1 ile ilgili az sayıda çalışma mevcut olup çalışmalarda sonuçlar farklılık göstermektedir. Bu çalışmada mesane ürotelyal karsinomlarda Cyclin D1ekspresyonunun prognostik faktörler ile ilişkisi araştırıldı.

Gereç ve Yöntemler: Çalışmaya Kafkas Üniversitesi Sağlık Araştırma ve Uygulama Hastanesi'nde TUR-M yapılmış 46 olgu dahil edildi. Olgulara dair genel bilgiler ve patoloji raporları hastane otomasyon sisteminden elde edildi. Hematoksilen&Eozin boyalı patoloji preparatlarından tümör içeren kesitler seçilip seçilen kesitlere ait bloklara Cyclin D1 primer antikoru ile immünohistokimyasal boyama manuel yöntem ile yapılmıştır.Boyanan kesitler ışık mikroskobunda nükleer ve sitoplazmik olarak ayrı ayrı 0-4 olarak skorlanarak değerlendirildi.

Bulgular: Olguların yaş aralığı 51-93 olup ortalama yaş 69.2±11.7'dir. Olguların 12'si (%26.1) kadın, 34'ü (%73.9) erkektir. Histopatolojik bulgular incelendiğinde olguların 29'unun (%63.0) düşük dereceli 17'sinin (%37.0) yüksek dereceli olduğu gözlenmiştir. Olguların 18'i (%39.1) invaziv, 28'i (%60.9) noninvaziv niteliktedir. Yapılan istatistiksel analizlerde invazyon gösteren tümörlerin non-invaziv tümörlere göre (pTa) anlamlı düzeyde yüksek dereceli olduğu dikkati çekmiştir (p= 0.007). Benzer şekilde ve invaziv tümörlerde lenfovasküler invazyon varlığı non-invaziv tümörlere göre anlamlı derecede daha fazla saptanmıştır (p=0.001). Nükleer cyclin

Abstract

Objective: Cyclin D1 is a protein that is involved in the regulation of the cell cycle and is encoded by the CCND1 gene. There are few studies on Cyclin D1 in the literature, and the results differ in the studies. In this study, the relationship between Cyclin D1 expression and prognostic factors in bladder urothelial carcinomas was investigated.

Materials and Methods: Forty-six patients who underwent TUR-M at the Kafkas University Health Research and Application Hospital were included in the study. General information about the cases and pathology reports were obtained from the hospital automation system. Tumor-containing sections were selected from the Hematoxylin and Eosin stained pathology slides, and immunohistochemical staining was performed manually with Cyclin D1 primary antibody on the blocks of the selected slides. Immunostained pathology slides were evaluated under light microscope by scoring 0-4 separately as nuclear and cytoplasmic scores.

Results: The age range of the cases was 51-93, and the mean age was 69.2±11.7. Twelve (26.1%) cases were female and 34 (73.9%) were male. It was observed that 29 (63.0%) of the cases were low-grade and 17 (37.0%) were high-grade. Eighteen (39.1%) of the cases were invasive and 28 (60.9%) were noninvasive. In the statistical analyzes, it was noted that invasive tumors had a significantly higher grade compared to non-invasive tumors (pTa) (p= 0.007). Similarly, the presence of lymphovascular invasion in invasive tumors was statistically <0.005 higher than that of in non-invasive tumors. (p=0.001). It was observed that

The study was approved by Ethics Committee of Kafkas University (Approval number: 2021.07.01/29). All research was performed in accordance with relevant guidelines/regulations, and informed consent was obtained from all participants.

D1 expresyonunun (p=0.003) invaziv olgularda anlamlı düzeyde daha yüksek olduğu izlenmiştir. Ayrıca düşük dereceli tümörlerde nükleer cyclinD1 ekspresyonu anlamlı düzeyde yüksek bulunduğu saptanmıştır (p=0.044).

Sonuç: Çalışma sonucunda mesane ürotelyal karsinomlu hastalarda Cyclin D1 ekspresyonun tümör derecesi ve invazyon durumu ile ilişkisi gözlenmiştir ancak Cyclin D1'in biyobelirteç olarak kullanılabilmesi için daha geniş olgu serilerinde çalışmalara ihtiyaç duyulmaktadır.

Anahtar Kelimler: mesane, Cyclin D1, üretelyal karsinom, immünohistokimya

nuclear cyclin D1 expression (p=0.003) was significantly higher in invasive cases. In addition, nuclear cyclinD1 expression was found to be statistically significantly higher in low-grade tumors (p=0.044).

Conclusion: As a result of the study, a relationship between Cyclin D1 expression and tumor grade and invasion status was observed in patients with bladder urothelial carcinoma, but studies with larger case series are needed to use Cyclin D1 as a biomarker.

Keywords: bladder, Cyclin D1, urothelial carcinoma, immunohistochemistry

INTRODUCTION

Bladder carcinoma is the fourth most common cancer in men and the seventh most common cancer in women (1). Despite advances in treatment, its prognosis still remains poor (2). In bladder carcinomas, mortality and recurrence rates increase as urothelial carcinomas progress from the superficial form to the invasive form (3). In 70% of patients, the tumor is limited to the lamina propria (4) Recurrence may occur in at least one of 50% of bladder tumors after treatment. Recurrence after treatment is partially related to the histological grade and depth of invasion (5). Although factors such as tumor diameter, grade and stage are routinely used to determine recurrence and prognosis, these factors are often not sufficient to determine the course of the tumor (6). Therefore, studies are continuing to understand the effectiveness of treatment methods by determining the recurrence

and prognosis in these tumors (6). The way to do this is through the use of effective markers showing recurrence and prognosis. One of these markers is Cyclin D1, that has been reported to be a marker that plays a very important role in the emergence, development and spread of bladder cancer (7).

Cyclins are the regulatory subunit of "Cyclins are the regulatory subunit of cyclin dependent kinases (CDK)" and provide control in the transition from one phase to another in different phases of the cell cycle (8). The transient emergence of cyclins controls the enzymatic activity of CDKs resulting in the formation of a cyclin/CDK complex. Cyclin AS is required for transition to S phase and G2 phase in mammalian somatic cells (9). While Cyclin D and E control the transition to G1, Cyclin B has assumed the role of an important controller of all mitosis (10). Their mutations cause the cell to get stuck in G2 phase. It plays a role in all stages of the disease in renal cell carcinomas and various soft tissue tumors (11).

The cell cycle consists of G1 (presentetic), S (DNA synthesis), G2 (premitotic) and M (mitotic) stages. The transition from G1 to S is believed to be the most important control point in the cell cycle (12). The progression of the cell from phase to phase during the cycle is controlled by cyclins, cyclin-dependent kinases (CDK) and their inhibitors (13). Cyclins are among the positive regulators of this cycle (14). Cyclins, together with their catalytic subunit, cyclin-dependent kinase (CDK), play an important role in the cell cycle (15). CDK inhibitors, which are negative regulators, regulate the activity of the Cylin-CDK complex (17). Disruption of cell cycle regulation plays a key role in the development and progression of malignant tumors (16). In normal tissues, cyclins are positive regulators of the cell cycle and are associated with cell proliferation (16). However, their expression in tumors is deregulated depending on the proliferation status of the tumor (17). Cyclin D1 overexpression is observed in many tumors including liver, breast, lung, head and neck tumors (18). Recent studies have shown that CylinD1 overexpression is also found in bladder urothelial carcinomas, and this is associated with low grade in many studies

Neoplastic expression can be detected in a wide variety of Cyclin D1/bcl-1 (=G2 M phase Cyclin) processes, including bladder urothelial cancers (19). For example, 50-70% of Mantle cell lymphomas have been demonstrated to overproduce this protein (19). In the G1-S phase transition, an increase in the amount

of cyclin D1 is observed. In a study conducted in lung cancer patients other than small cell lung cancers, it was determined that p53 protein and CyclinD1 have a very significant interaction(20). It has been reported that Cyclin D1 is effectively used in the follow-up of pathogenesis and prognosis in these tumors and in cases with diffuse alveolar destruction (21). The increase in this protein is an important marker indicating poor prognosis (22). Tumor grade and stage are important prognostic indicators in urothelial tumors of the bladder. Molecular changes associated with bladder carcinoma contribute to the determination of prognosis. Therefore, in this study, we aimed to evaluate the relationship of Cyclin D1 marker with tumor stage, grade and lymphovascular invasion in patients who underwent transurethral resection (TUR) for bladder urothelial carcinoma.

MATERIAL AND METHODS

Prior to the study, approval was obtained from the Non-Invasive Studies Ethics Committee of the Faculty of Medicine of Kafkas University, dated 01.07.2021 and numbered 07. Forty-six patients who underwent TUR-M at the Kafkas University Health Research and Application Hospital were included in the study. General information about the cases and pathology reports were obtained from the hospital automation system. Tumor-containing sections were selected from the H&E stained pathology slides, and the blocks of the selected slides were immunohistochemically stained with Cyclin D1 by a manual method (Clone SP4, ThermoScientific, USA) primary antibody as indicated below.

Sections were taken from paraffin blocks on a 3-4 micron thick adhesive slide. Sections were kept in an oven at 56 degrees overnight. The next day, the sections were kept in three separate xylenes for 5 minutes. Then, they were kept in graded alcohols for 5 minutes and washed in distilled water for 1 minute. They were boiled in 10% citrate buffer (Sigma-Aldrich, USA, Ph6.0) solution for 10 minutes. The lid of the vessel containing the boiled slides was opened and kept at room temperature for 20 minutes. The sections were rinsed with distilled water and kept in 10% hydrogen peroxide solution for 10 minutes, then washed again in distilled water and kept in a W block (ThermoScientific, USA) for 5 minutes. At the end of the period, the primary antibodies diluted at a ratio of 1:50 were dropped by shaking the W block on the sections without washing. Antibodies were incubated for 60 minutes. After incubation, washing was done in distilled water for 10 minutes. Then, it was passed to the secondary antibody stage and kept in biotin (ThermoScientific, USA) solution for 20 minutes, washed in distilled water for 5 minutes and kept in streptavidin (ThermoScientific, USA) solution for 20 minutes. After washing in distilled water for 5 minutes, it was incubated in DAB chromogen (ThermoScientific, USA) for 7 minutes and washed. Finally, after 5 minutes of staining in Mayer's hematoxylin (Bio-Optica, Italy), it was passed through alcohol and xylene and closed with a mount.

Immunohistochemically stained sections were evaluated nuclear and cytoplasmic separately under the light microscope (Olympus BX51 BF/DF) by scoring "0: no staining, 1: mild staining, 2: moderate staining, 3: strong staining". SPSS 15.0 package program was used for statistical analysis (SPSS Inc. Released 2006. SPSS for Windows, Version15.0. Chicago, SPSS Inc.). The conformity of the immunohistochemical staining scores to the normal distribution was evaluated with the Kolmogorov Smirnov and Shapiro Wilk tests. Immunohistochemical staining scores and tumors were compared according to their pT stage and categorized as invasive/noninvasive in analyzes performed using Mann Whitney U, Kruskal Wallis tests and T test for independent samples in the 95% confidence interval. In addition, low/high tumor grade and presence of lymphovascular invasion were also compared with the staining scores.

RESULTS

The mean age of cases was 69.2±11.7. Twelve (26.1%) cases were female and 34 (73.9%) were male. It was observed that, 29 (63.0%) of the cases were low-grade and 17 (37.0%) were high-grade. Eighteen (39.1%) of the cases were invasive and 28 (60.9%) were noninvasive. In the pathological stage evaluation, it was noted that 28 (60.9%) cases were pTa, 11 (23.9%) cases were pT1 and 7 (15.2%) cases were pT2. While lymphovascular invasion was detected in 6 (13.0%)

cases; it was not observed in 40 (87.0%) cases. In the statistical analyzes performed, it was noted that invasive tumors had a significantly higher grade compared to non-invasive tumors (pTa) (p= 0.010). Similarly, the presence of lymphovascular invasion in invasive tumors was significantly higher than in non-invasive tumors (p=0.009) (Table-1).

Table 1. Patient and tumor characteristics

Variable	n (%)
Age	69.2±11.7
Gender	
Woman	12 (26.1)
Male	34 (73.9)
Invasive	18 (39.1)
Noninvasive	28 (60.9)
Grade	
Low grade	29 (63.0)
High grade	17 (37.0)
Tumor Classification	
pTa	28 (60.9)
pT1	11 (23.9)
pT2	7 (15.2)
pT3	0 (0)
pT4	0 (0)
Presence of lymphovascular Invasion	6 (13)

Table 2. Nuclear and cytoplasmic cyclin D1 expression scores

Variable	n (%)
Nuclear cyclin D1 expression	
0 (no staining)	9 (19.6)
1 (mild staining)	15 (32.6)
2 (moderate staining)	11 (23.9)
3 (strong staining)	11 (23.9)
Cytoplasmic cyclin D1 expression	
0 (no staining)	30 (65.2)
1 (mild staining)	16 (34.8)
2 (moderate staining)	0 (0)
3 (strong staining)	0 (0)

Nuclear and cytoplasmic cyclin D1 expression is demonstrated in figures 1-3 and scores are presented in Table 2. The staining scores were not distributed normally (p=0.000 on both Kolmogorov Smirnov and Shapiro Wilk tests). The interquartile range for nuclear cyclin D1 scores were calculated as 1 as well as cytoplasmic cyclin D1 scores. It was detected that neither age nor gender showed statistical significance with nuclear cyclin D1 expression (p=0.909, p=0.458 respectively) and cytoplasmic cyclin expression (p= 0.928, p=0.905 respectively). In the analyzes, it was observed that nuclear cyclin D1 expression (p=0.002) was statistically significantly higher in invasive cases. However, the same significance could not be demonstrated between cytoplasmic cyclinD1 (p=0.428) expression and invasion. There was also a statistical significance between pT stage and nuclear cyclin D1 expression (p=0.011). In addition, as in invasion, no correlation was found with cytoplasmic expressions (p= 0.730). With the Mann Whitney U test, nuclear cyclinD1 (p=0.044) expression was found to be statistically significantly higher in low-grade tumors, but the same relationship could not be shown in cytoplasmic expression (p= 0.562). It was noted that the nuclear (p=0.112) and cytoplasmic cyclin D1 (p=0.937) expression in the mannWhitney U test for lymphovascular invasion did not show a statistically significant difference.

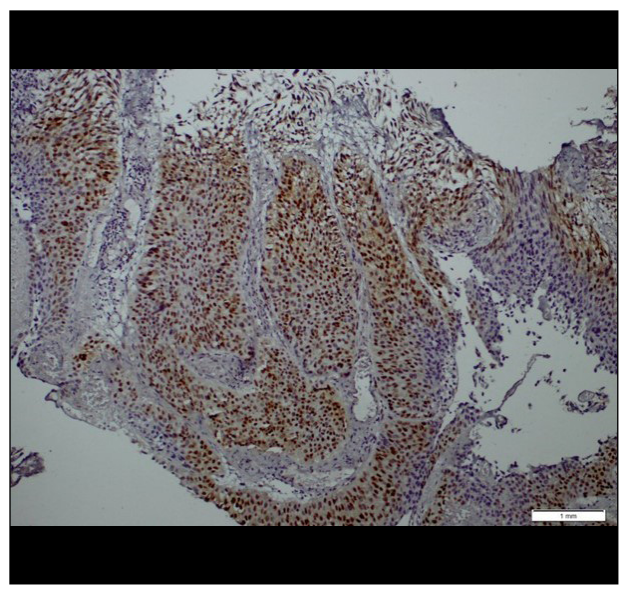

Figure 1. Moderate cytoplasmic and nuclear cyclin D1 staining, 100x

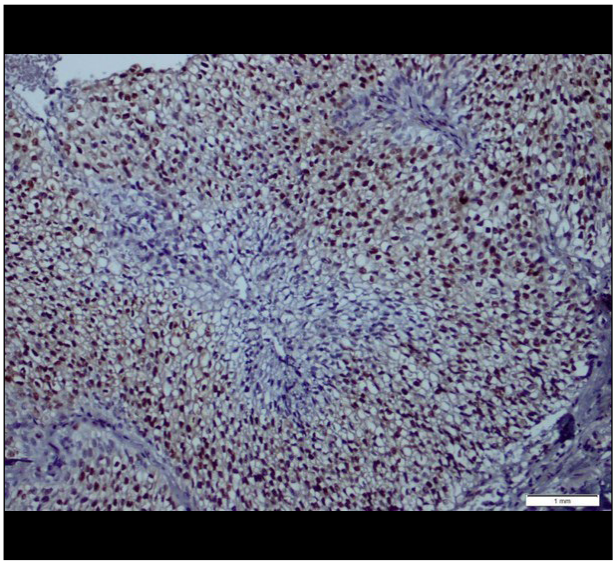

Figure 2. Mild cytoplasmic and strong nuclear cyclin D1 staining, 200x

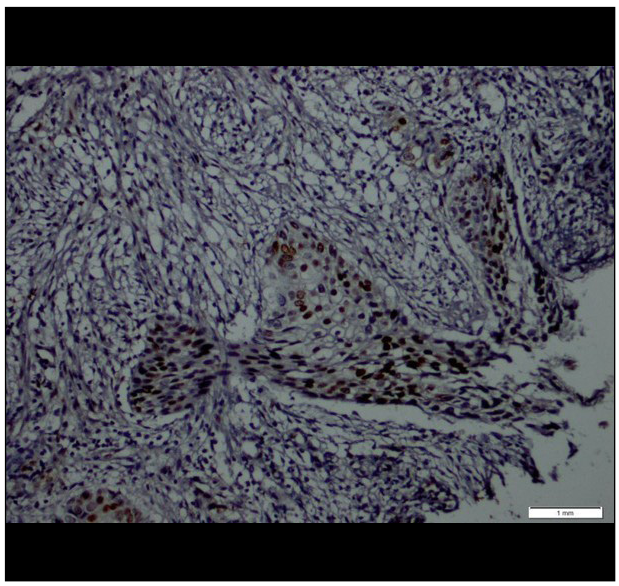

Figure 3. No cytoplasmic cyclin D1 staining and moderate-strong nuclear cyclin D1 staining, 400x

DISCUSSION

Conventional prognostic factors provide important information in evaluating the prognosis of bladder cancer (23). However recently, new markers related to cell cycle and apoptosis mechanisms, which are thought to provide more objective data in evaluating the prognosis and clinical course of bladder tumors, have been studied. Among the conventional prognostic factors, staging is the most important prognostic factor in bladder cancer (24). While 5-year survival is more than 75% in tumors with only lamina propria invasion, it decreases to 40% in muscularis propria invasion and 20% in perivesical adipose tissue invasion (25). While recurrence rate and rate in progression in low grade urothelial carcinoma are is 64% and 10.5%, respectively; recurrence rate in high grade urothelial carcinoma is 56.4% and risk of progression is 27% (26).

In some studies, no relationship was found between cyclin D1 and stage and grade, but it was reported that patients with high Cyclin D1 expression had a long disease-free survival and recurrence was higher in those with low Cyclin D1 expression (26). When cell cycle-related genes are altered, they can cause neoplastic transformation. Cyclin D1 is one of them that plays a role as an oncogene for various neoplasms (27). This is generally said to occur with increased expression and amplification of mRNA (27). In recent studies, it has been determined that high expression of exogenous Cyclin D1 in breast cancer cells inhibits rather than increases the growth of breast cancer cells. These also support the double-acting role of Cyclin D1 in the cell cycle (28).

By defining the signal transmission pathways of molecules, it has facilitated our understanding of processes such as cellular life, metastasis, and invasion in tumors (29). Apoptosis, which occurs as a result of mutation, plays an important role in carcinogenesis, metastasis and invasion of the disease (29). Many studies are carried out to determine the relationship of genes with epithelial mesenchymal transition, apoptosis and angiogenesis in bladder tumors. Cyclin D1 is one of these studies. It has been reported that staining with a good prognosis is high, especially in studies performed to determine prognosis (29). In the staining performed by Sayar (2016) on 149 cases, the pT stage of Cyclin D1 was found to be significantly higher in TaT1, low grade, cases without recurrence and progression, and the first pT stage T2-T4 in high grade cases (1).

Khabaz et al. (2016); found a significant correlation with the stage and invasion of the tumor with Cyclin

D1 staining performed on 128 bladder tumor cases. They reported that there was a high degree of staining in low-stage bladder tumors (30). Amer et al. (2019); In a study of Amer et al, the authoprs examined the correlation of CyclinD1 and p53 expression in bladder tumors in 90 cases. According to the staining results, weak expression was observed in high-grade bladder tumors (31).

Regarding Cyclin D1, it was expressed in 85% of the non-neoplastic urothelium and 76.2% (P>0.05) of the malignant group in the current study. Our results are in line with Khabaz et al., 2016, who reported similar frequency of Cyclin D1 immunoreactivity in bladder tumors (51.6%) and normal bladder tissue (50%) (30). A study by in 2007 reported equal intensity of Cyclin D1 expression in the non-neoplastic group. However, in other studies (31), higher Cyclin D1 protein expression was reported in UBC and endometrial carcinoma compared to adjacent normal tissue. In other studies (30), complete absence of Cyclin D1 has been reported in normal urothelium and other tissues such as colonic and gastric mucosa (32) expressed in the carcinoma group.

In the current study, high expression of Cyclin D1 is associated with the early-stage group (P=0.031), which is consistent with several studies (33), as well as lymph node involvement and MIBC. The present signs showed no bilharzial invasion (P=0.001), consistent with a study by (34) that showed a more advanced occurrence of UBC disseminated with schistosomiasis in all elevated IRS Cyclin D1 cases (20/20). Positive prognostic uses of Cyclin D1 UBC (33).

The favorable prognostic effect inferred from Cyclin D1 overexpression is attributed to early-stage evidence that cell proliferation with no tumor invasion or metastasis is a necessary step and low Cyclin D1 expression may be, as suggested by Guang and Tian (2015). A surrogate for other genetic events in the same cells ultimately drives cell growth and leads to a worse prognosis (35). In addition, the phenotype of Cyclin D1 was associated with the extent of cancer progression and the degree of invasiveness. Altered expression of Cyclin D1 can lead to changes in the biological behavior of transformed cells, such as growth, proliferation, invasion and metastasis (36). The inverse correlation between Cyclin D1 expression and poor prognostic parameters has not been reported only in urothelial carcinoma; it is also found in gastric carcinoma, laryngeal squamous cell carcinoma and invasive breast carcinoma among other tumors (37).

Limitations

Due to the fact that the patients were treated in different centers and/or lacked follow- up, recurrence and survival could not be evaluated with data.

CONCLUSION

In our study, recurrence was observed to be higher in T1 stage compared to Ta stage tumors but it was not found to be statistically significant. Consistent with the general information, it was observed that the recurrence rate increased significantly in the T2 stage. Low-grade carcinomas were found to recur significantly more than high-grade carcinomas. It was observed that high-grade carcinomas also recurred more than low-grade carcinomas, but it was not statistically significant.

New potential prognostic markers are being identified that will provide important information in detecting the clinical course of bladder cancer and in determining the recurrence or progression of patients. We know that some molecules play very important roles in cell regulation disorders and that altered expressions of these molecules may be effective in tumor progression. In this study, a relationship between Cyclin D1 expression and tumor grade and invasion status was observed in patients with bladder urothelial carcinoma, but studies with larger case series are needed to use Cyclin D1 as a biomarker.

Conflict of Interest

The authors declare to have no conflicts of interest.

Financial Disclosure

The authors declared that this study has received no financial support.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

Ethical Approval

The study was approved by Ethics Committee of Kafkas University (Approval number: 01.07.2021/29).

The study protocol conformed to the ethical guidelines of the Helsinki Declaration.

Author Contributions

Conception and design: Beşeren H, Adalı Y, Data acquisition: Ezer M, Data analysis and interpretation: Ertürk F, Drafting the manuscript: Beşeren H, Critical revision of the manuscript for scientific and factual content: Beşeren H, Statistical analysis: Beşeren H, Supervision: Beşeren H, Adalı Y.

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