



## ORJİNAL MAKALE / ORIGINAL ARTICLE

Balıkesir Sağlık Bilimleri Dergisi / BAUN Sağlık Bil Derg  
Balıkesir Health Sciences Journal / BAUN Health Sci J  
ISSN: 2146-9601- e ISSN: 2147-2238  
Doi: <https://doi.org/10.53424/balikesirsbd.1241429>



### Clinicopatological Examination of Waldeyer's Ring Lymphomas: A Cross-Sectional Study in Turkey

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*Geliş Tarihi / Received: 24.01.2023, Kabul Tarihi / Accepted: 13.02.2023*

#### ABSTRACT

**Objective:** Waldeyer's ring (WR) is the second most common site of extranodal lymphomas. Its clinical course may resemble those of nodal lymphomas. We evaluated the histopathological and clinical findings, Epstein-Barr virus (EBV) presence, and overall survival of WR lymphomas diagnosed in our department. **Materials and Methods:** Retrospectively, 32 patients were included. The demographic and clinical data of the patients and lymphoma types, the presence of EBV [EBV early RNAs (EBER)] by chromogenic in-situ hybridization (CISH) method were examined. Kaplan-Meier analysis was used for the survival rate. A P value less than 0.05 was considered statistically significant. **Results:** 23 of 32 cases were male. The mean age was 57.97±16.15 years. The preliminary clinical diagnosis was carcinoma in 46.9% of the cases. Tonsil and nasopharynx had equal proportions as lymphoma origin. Only 3 (9.4%) cases are classic Hodgkin lymphoma (CHL). 16 cases (50.0%) were diffuse large B-cell lymphoma (DLBCL). EBER was detected only in tonsillar CHL cases. The survival of patients with nasopharyngeal lymphoma was shorter than those of tonsillar origin (p=0.037). **Conclusion:** DLBCL is the most common lymphoma type of this localization. EBER positivity has been detected only in CHL. Survival is lower in lymphomas originating from the nasopharynx.

**Keywords:** Non-Hodgkin Lymphoma, Nasopharynx, Tonsil, Hodgkin Lymphoma.

### Waldeyer Halkası Lenfomalarının Klinikopatolojik İncelenmesi: Türkiye'de Kesitsel Bir Çalışma

#### ÖZ

**Amaç:** Waldeyer halkası (WH), ektranodal lenfomaların ikinci sıklıkta görüldüğü yerdir. Klinik seyri nodal lenfomalara benzeyebilmektedir. Bu çalışmada WH lenfomalarının histopatolojik ve klinik bulgularını, Epstein-Barr virüsü (EBV) varlığını, genel sağ kalım verilerini değerlendirdik. **Gereç ve yöntem:** Çalışmamız retrospektiftir. WH lenfoma tanılı 32 hastaya ait demografik, klinik veriler, lenfoma tipleri ve kromojenik in-situ hibridizasyon yöntemi (KİSH) ile EBV (EBER) varlığı incelendi. Tanımlayıcı istatistikler yapıldı. Hayatta kalma oranı için Kaplan-Meier analizi kullanıldı. P değerinin 0.05'ten küçük olması anlamlı kabul edildi. **Bulgular:** Çalışmamızdaki 32 vakanın 23'ü erkek, 9'u kadın olup, hastaların yaş ortalaması 57.97±16.15'di. Vakaların %46.9'unda klinik ön tanı karsinomdu. Lenfoma yerleşimi tonsil ve nazofarenkste eşitti. Vakaların sadece 3'ü klasik Hodgkin lenfoma (KHL), diğerleri Non-Hodgkin lenfoma (NHL) tanılıydı. NHL tanılı olguların 16'sı diffüz büyük B hücreli lenfoma (DBBHL) idi. DBBHL'ların %50.0'si germinal merkez B (GMB) hücre kökenli iken, %50.0'si non-GMB kökenli idi. EBER pozitifliği sadece tonsil KHL vakalarında izlendi. Ann Arbor evreleme sistemine göre vakaların %58.3'ünün evresi I-II idi. Nazofaringeal kaynaklı lenfoma hastalarının sağ kalımı tonsil kaynaklı olanlardan anlamlı olarak kısa idi (p=0.037). **Sonuç:** WH lenfomaları klinik olarak en sık karsinomlar ile karıştırılmaktadır. Tonsil ve nazofarenks kaynaklı olanlar oranca birbirine yakındır. EBER pozitifliği sadece KHL'larda tespit edilmiştir. Nazofarenksten kaynaklanan lenfomalarda sağ kalım daha düşüktür.

**Anahtar Kelimeler:** Non-Hodgkin Lenfoma, Waldeyer Halkası, Nazofarenks, Tonsil, Hodgkin Lenfoma.

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**Bu makaleye atf yapmak için / Cite this article:** Özdemir, Ç., Eryeğen, N., Yavaşoğlu, F., Ak, M.O., Kuzu, S., Duran, M., Belen Aydoğmuş, B., Çetinkaya, Z.B., Bucak, A., & Şenol, Y. (2023). Clinicopatological examination of waldeyer's ring lymphomas a cross-sectional study in Turkey. *BAUN Health Sci J*, 12(1): 81-88.

<https://doi.org/10.53424/balikesirsbd.1241429>



BAUN Health Sci J, OPEN ACCESS <https://dergipark.org.tr/tr/pub/balikesirsbd>

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## INTRODUCTION

Lymphoma is one of the most common malignancies worldwide. However, its distribution varies geographically around the world. Besides clonal malignancies, lymphomas are divided into two groups, nodal and extranodal, according to the lymphoid tissue from which they originate (Swerdlow, 2017). Waldeyer's ring (WR) is one of the extranodal lymphoid tissue regions, and the tissues forming this ring are the nasopharynx, palatine tonsil, tongue root, and oropharyngeal wall. At the 1971 symposium, WR was accepted as a separate in the ageing of patients with lymphoma (Hoppe et al., 1978). Extranodal lymphomas in the human body are the second most common place, and head and neck region extranodal lymphomas are the most common (d'Amore et al., 1991; Mian et al., 2014). The majority of WR lymphomas originate from the nasopharynx and tonsils. The most common type is non-Hodgkin lymphoma (NHL), and the subgroup is diffuse large B-cell lymphoma (DLBCL) (Teh et al., 2014). It has also been reported very rarely in Hodgkin lymphoma and T-cell lymphomas. (Wang et al., 2021). *Epstein-Barr virus* (EBV) is a herpes virus and is known to be the cause of lymphoid neoplasms. EBV positivity in lymphomas differs between countries (O'Malley et al., 2019). The lymphoma classification and the addition of new provisional entities are periodically revised by the World Health Organization (WHO) (Alaggio et al., 2022). Extranodal lymphomas have stage and clinical differences compared to nodal lymphomas (de Leval et al., 2012).

Studies on WR lymphomas are scarce worldwide, and they are given in the form of case reports in our country. In our research, the research evaluates the distribution of WR lymphomas by anatomical region, identified immunophenotypic subgroups, EBV status, clinical stage, survival data, and other clinicopathological parameters.

## MATERIALS AND METHODS

### Study type

The retrospective study included 32 patients with WR out of 352 patients diagnosed with lymphoma in the pathology laboratory between 2017-2022. The age, gender and clinical information of the patients were obtained from the hospital's digital information system. The pathologic diagnosis of patients was reevaluated, the results of the immunohistochemical studies, and the EBV [EBV early RNAs (EBER)] status of the patients detected by the chromogenic in-situ hybridization (CISH) method for EBV were recorded from the pathology reports. Lymphomas have been reclassified immunophenotypically according to the recent revision of WHO (Alaggio et al., 2022). DLBCL was divided into germinal center B cell lymphomas (GMB) and non-GMB cell lymphomas based on

immunohistochemically CD10, MUM1, Bcl6 expression according to Hans algorithm in immune phenotypic classification (Hans et al., 2004). The Ann Arbor staging system was used for NHL and Hodgkin's disease (Shi et al., 2019).

The patients' clinical preliminary diagnosis, B symptoms, clinical stage, histopathological diagnoses, EBER positivity, and overall survival were recorded too.

### Statistical analysis

PASW Statistics 18.0 program was used for statistical analysis. Descriptive statistics evaluated categorical data as percentage and frequency and continuous data as mean and standard deviation (mean±sd). Kaplan-Meier survival analysis was used for the survival rate. The log-rank test was used to compare the survival curves. Cox regression analysis was applied to evaluate the effect of various factors on survival time. p value less than 0.05 was considered statistically significant.

### Ethical considerations

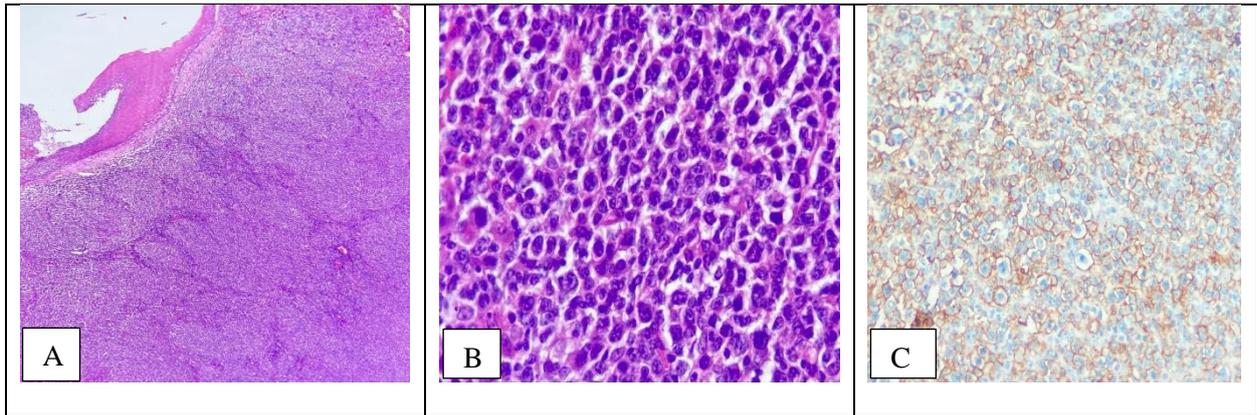
The study was approved by Clinical Research Ethics Committee number: 2022/401 dated August 5, 2022.

## RESULTS

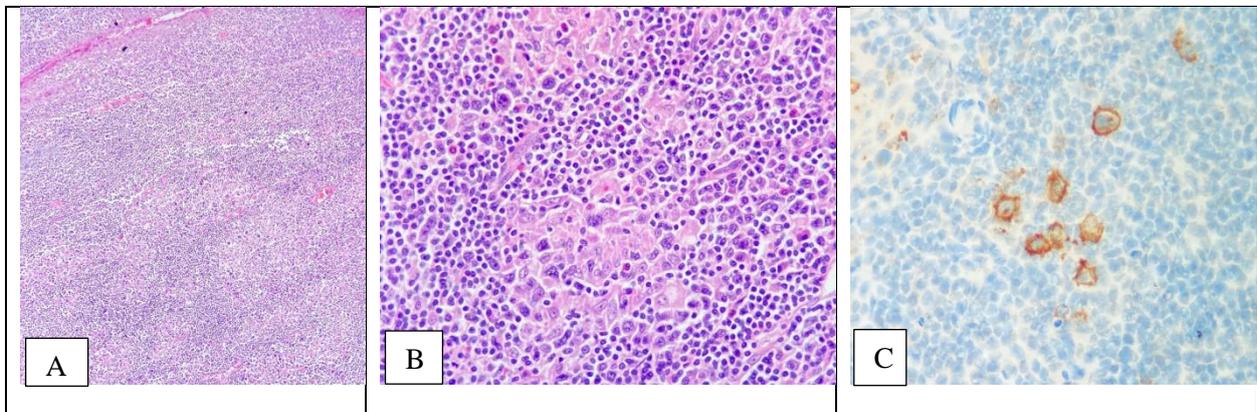
Thirty-two patients were included in the study. The mean age of patients was 57.97±16.15 years, the oldest patient was 88 years old, and the youngest patient was 22 years old. 23 (71.9%) were male, and 9 (28.1%) were female. The tissue biopsies were planned 46.9% of patients with the pre-diagnosis of carcinoma and 34.4% of patients with PR diagnosis of lymphoma. While 17 (53.1%) lymphomas were the tonsillar origin, 15 (46.9%) lymphomas were nasopharyngeal. 90.6% of lymphomas were NHL (Figure 1) and 9.4% were Hodgkin lymphoma (HL) (Figure 2). The NHL subtype was diagnosed most frequently (50%) with DLBCL and (18.8%) DLBCL/high-grade B-cell lymphoma classified by MYC and BCL2 rearrangements. According to Hans' algorithm, 50% of DLBCLs were GMB cells and 50% were non-GMB. EBER positivity (Figure 3) was detected only in tonsillar Classical Hodgkin lymphoma (CHL) cases, the remaining cases were EBER negative. Other findings of the patients are summarized in Table 1.

B symptoms are present in only 9 (28.1%) of the patients. According to the Ann Arbor staging system, 58.3% of the cases were stages I-II. The overall survival of 32 patients is shown in Figure 1. 50.0% (n=16) of the patients died during the follow-up period. The median life expectancy was 22.13 months. Estimated 1-year survival rates were 57.5% (Figure 1).

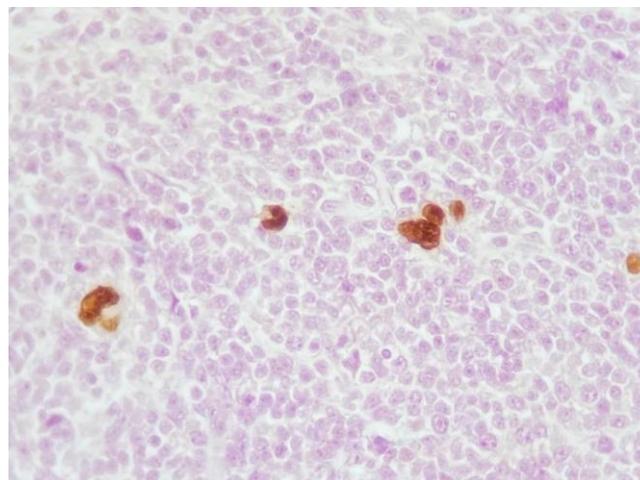
The median life expectancy in women was 10.33 months and the 1-year survival rate was 44.3%. The median life expectancy in males was 42.77 months and the 1-year survival rate was 62.9% (Figure 2). There was no statistically significant difference in survival between men and women (p=0.398).



**Figure 1.** Lymphoma infiltration with mass just below the surface epithelium in tonsillar lymphoma, diffuse large B-cell lymphoma (A) (HE, x40). Lymphoma cells are large, with prominent nucleoli, with eosinophilic cytoplasm (B) (HE, x400). Cytoplasmic diffuse strong reaction in lymphoma cells to CD20 antibody (C) (X200).



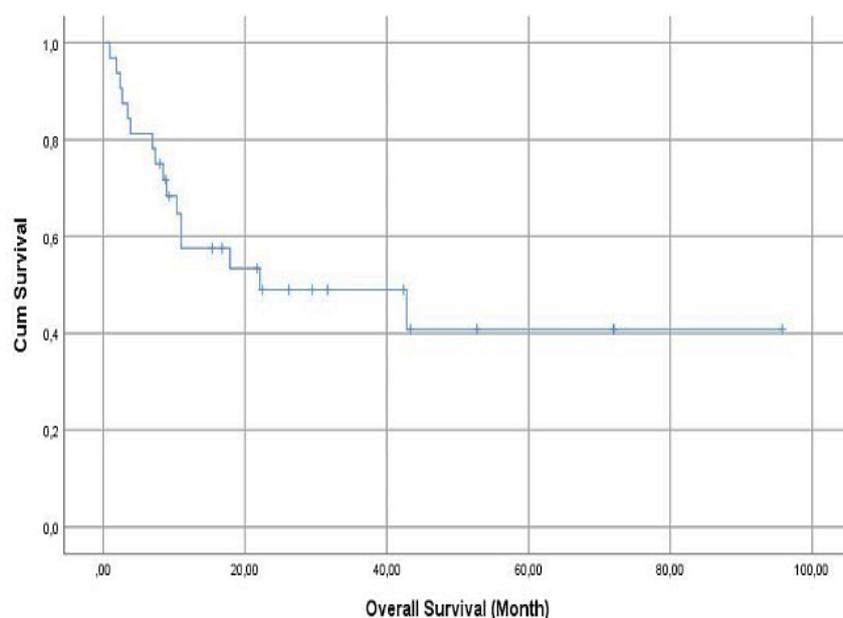
**Figure 2.** In tonsillar lymphoma, infiltration consisting of Hodgkin cells in a non-neoplastic inflammatory background just below the surface epithelium, classical Hodgkin lymphoma, mixed cellular (A) (HE, x100). Hodgkin cells are large cells within lacunae and with red nucleoli, some are binucleated and these are Reed-Sternberg cells (B) (HE, x400). Membrane and golgi zone positivity in lymphoma cells to CD30 antibody (C) (X400).

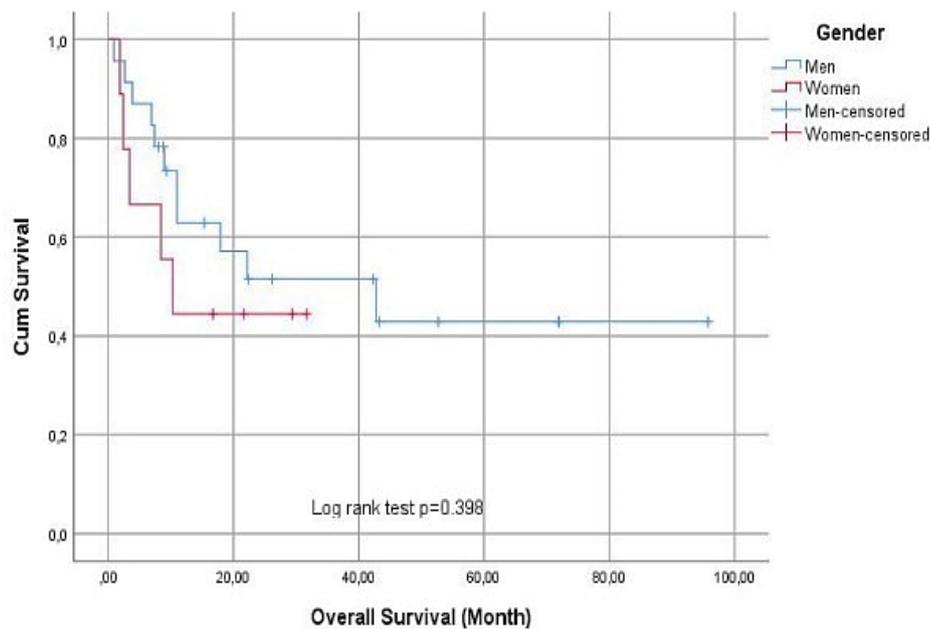


**Figure 3.** Epstein Barr virus early RNAs, EBER nuclear strong positivity in Hodgkin lymphoma cells (x400).

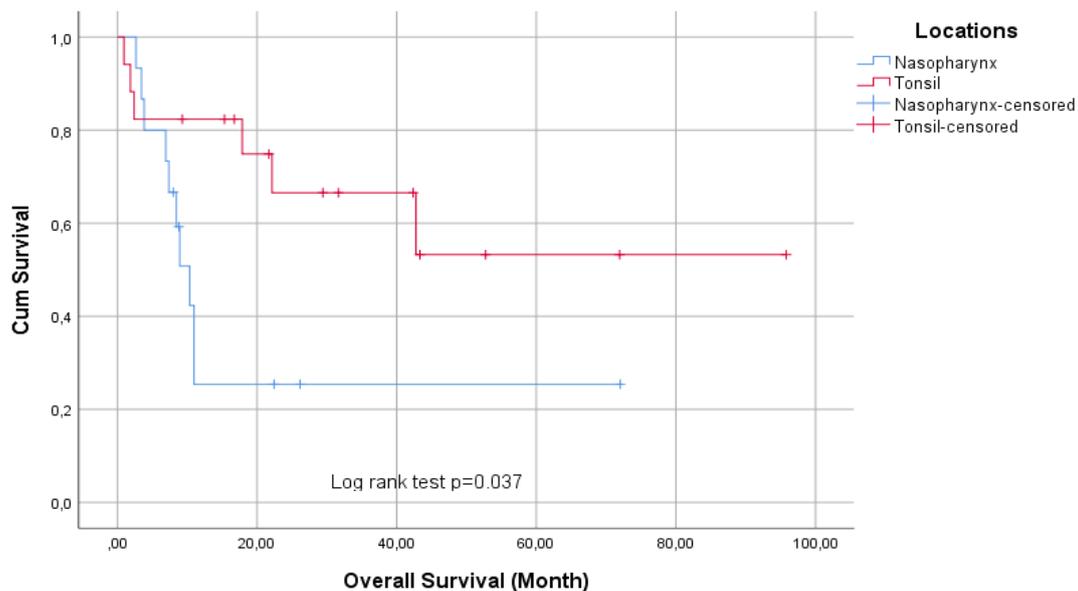
**Table 1. Gender, pre-biopsy clinical diagnosis, lymphoma subtypes of the patients (n=32).**

Variables	n	%
<b>Gender</b>		
Male	23	71.90
Female	9	28.10
<b>Total</b>	32	100.00
<b>Clinical pre-diagnosis</b>		
Carcinoma	15	46.90
Lymphoma	11	34.40
Tonsillar hypertrophy	3	9.40
Adenoid vegetation	1	3.10
Inverted papilloma	1	3.10
Lymphoma. Metastasis	1	3.10
<b>Lymphoma origin</b>		
Tonsil	17	53.10
Nasopharynx	15	46.90
<b>Lymphoma diagnosis group</b>		
Non-Hodgkin lymphoma	29	90.60
Hodgkin lymphoma	3	9.40
<b>Diagnostic subtype</b>		
Diffuse large B-cell lymphoma	16	50.00
Diffuse large B-cell lymphoma/high-grade B-cell lymphoma classified by MYC and BCL2 rearrangements according	6	18.80
Mantle Cell Lymphoma	3	9.40
Small lymphocytic lymphoma	2	6.30
Marginal zone lymphoma	2	6.30
Classical Hodgkin lymphoma. lymphocyte-rich	1	3.10
Classical Hodgkin lymphoma. mixed cellular	2	6.20

**Figure 4. Overall survival analysis results.**



**Figure 5. Comparison of overall survival outcomes by gender.**



**Figure 6. Comparison of overall survival outcomes by lymphoma location**

9 (60%) of 15 patients with nasopharyngeal lymphoma died, and 6 (35.3%) of 17 patients with tonsillar lymphoma died. In nasopharyngeal lymphomas, the mean life expectancy was 24.02 months, the median survival time was 10.33, and the 1-year survival rate was 25.4%. The mean survival rate in tonsillar lymphomas was 60.18 months and the 1-year survival rate was 82.4% (Figure 3). There was a statistically significant difference between the survival of those with nasopharyngeal and tonsillar lymphoma ( $p=0.037$ ).

As a result of Cox Regression analysis, the risk of death in nasopharyngeal lymphomas was found to be 2.899 (95% Confidence interval 1.020-8.242) times more than in tonsillar lymphomas ( $p=0.046$ ).

#### DISCUSSION

Lymphoma classification is based on morphological, immunophenotypic and genetic information. According to this, all lymphomas are divided into two main groups as HL and NHL (Swerdlow, 2017). NHLs are a group with a wide variety of diagnoses.

Extranodal NHLs most commonly originate from WR after the gastrointestinal tract (Clarke et al., 2019; Swerdlow, 2017). HL originating from WR is rare (Swerdlow, 2017). As in our study, the rate of WR lymphomas among all lymphomas is around 10% (d'Amore et al., 1991). Considering the gender distribution of WR lymphomas, the female and male ratios are close, but it is more common in females (Ma et al., 2020; Teh et al., 2014). However, in our study, the incidence rate in men was considerably higher than in women's.

The most common location of lymphomas in WR is tonsil, and tonsil location rates are over 50% (Ma et al., 2020; Teh et al., 2014). Although lymphomas most commonly originate from the tonsil in our study, the nasopharynx was the most common site. Lymphoma distributions may show geographical differences and the differences in our study may be due to this.

WR lymphomas can show various clinical signs, and biopsy can be indicated with various preliminary diagnoses. Similar to previous studies, biopsies were performed with a preliminary diagnosis of carcinoma in both nasopharyngeal and tonsillar lymphomas in our study (Kaygusuz et al., 2008). WR lymphomas are most often confused with carcinoma clinically.

In nodal lymphomas, clinically, the presence of B symptoms is relatively higher than in the extranodal lymphomas (Shi et al., 2019). In our study, similar to other studies, the number of patients with B symptoms was less than patients with B symptoms (Ma et al., 2020). It may also be due to the earlier clinical stages of these patients. As in our study, clinical stages are usually III-IV in B-cell lymphomas of WR (Ma et al., 2020). HL's clinical stage mostly ranges from I to II (Qin et al., 2018).

The most common type of lymphoma originating from the nodal or extranodal is NHL with DLBCL subtype (Monabati et al., 2016; Sun et al., 2012). Similarly, in the current study, NHL was the most common type, and DLBCL was the most common subtype in our patients. However, in one of the studies in which more cases were included in the literature, follicular lymphoma was observed in WR with the second frequency after DLBCL (Ma et al., 2020). However, our study did not have follicular lymphoma in the tonsil and nasopharynx. Our study's second frequency was DLBCL/high-grade B-cell lymphoma classified by MYC and BCL2 rearrangements. High-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements is classified as DLBCL/high-grade B-cell lymphoma MYC and BCL2 rearrangements according to the new WHO revision (Alaggio et al., 2022).

DLBCL is divided into two as GMB cell and non-GMB depending on CD10, MUM1, and BCL6 expression according to the Hans algorithm in the immunophenotypic classification (Hans et al., 2004). In many studies, regardless of the anatomical region, extranodal DLBCL categorized according to the Hans

algorithm, is a highly non-GMB immunophenotype (Ma et al., 2020). However, in the study by de Laval et al., in DLBCL lymphomas of WR origin, GMB immunophenotype was found frequently (de Laval et al., 2012). In our study, we found both immunophenotypes equally.

CHL is rare in WR and accounts for approximately 1% of all WR lymphomas (H. Völker et al., 2020). In our study, the rate of CHL among WR lymphomas was 9.4%, which is quite high compared to other studies. The most common localization of CHL is variable. In some studies, it is located in the nasopharynx (H. U. Völker et al., 2020), and in the tonsil in some studies (Iyengar et al., 2010). In the study, two of 3 cases originated from the tonsil, and one from the nasopharynx.

EBV is an oncogenic virus from the herpes virus family. It has been proven to be associated with lymphoid malignancies in children and adults. Worldwide geographical differences describe the connection between lymphoma types and EBV positivity (Herbst et al., 1992). Although EBER positivity is variable in nodal or extranodal B-cell lymphomas, it is quite low (Swerdlow, 2017). In a study including 65 cases, EBER was found in only 2 cases in the WR B cell lymphomas (Ma et al., 2020). Our study showed no EBER-positive cases in B-cell lymphoma cases. EBER positivity is around 30% in extranodal HL of the head and neck region. Our study detected EBER positivity in 2 of the 3 HL cases. Contrary to some studies in the literature (Quiñones-Avila et al., 2005) our EBER-positive cases were located in tonsil region.

According to a previous study, life expectancy was found to be shorter in lymphomas originating from the nasopharynx than in the tonsils (Teh et al., 2014).

## CONCLUSION

Lymphomas show geographical differences in terms of anatomical region and immunophenotypic distribution. WR lymphomas constitute only 10% of all lymphomas.

WR lymphomas are often confused with carcinomas clinically. Although it has been reported that it is most common in the tonsil, in our study, lymphomas originating from the nasopharynx were found close to those originating from the tonsils. Although DLBCL is the most common type of lymphoma, CHL has been detected at a high rate, according to many studies. EBER was detected only in CHL cases. Survival is lower in lymphomas originating from the nasopharynx. Our study is the first study on this subject in Turkey.

## Conflict of Interest

The author declares no potential conflicts of interest concerning this article's research, authorship and/or publication.

**Author Contributions**

**Plan, design:** CO, MOA; **Material, methods and data collection:** YS, CO, MOA, NE; **Data analysis and comments:** YS, CO, MOA, FY, SK, MD, BBA, ZBC, AB; **Writing and corrections:** CO, MOA.

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