



## Research

# Regional Variation of Non-Hodgkin's Lymphoma (NHL) in Mongolia and its Association with Ki-67 Expression

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### Abstract

**Background:** The prevalence of non-Hodgkin's lymphoma (NHL) varies worldwide in association with demographic and environmental factors. The analysis of these associations in Asia, Africa, and less developed countries is limited by low absolute numbers and unknown etiologic factors such as in Mongolia. The geographic variations in NHL incidence and mortality rates may induce by differences in case ascertainment and registration, or disease diagnosis and classification. The interpretation of NHL patterns and trends remains difficult. Therefore, an attempt was made to test the correlation between Ki-67 expression and clinical parameters on one hand, and geographical or ethnic differences on the other.

**Research purpose:** The objectives of this study are to examine the geographic distribution of non-Hodgkin's disease more in detail for high incidence Mongolian prefectures, and to evaluate the association between the distribution of NHL and Ki-67 expression.

**Methods:** Expression of Ki-67 was examined using an immunohistochemical technique in archival paraffin-embedded sections taken from (n=35) both National pathology center of Mongolia and Etemo clinic previously. Geo-processing was conducted with the aid of the software R Studio [under the Mapping plots] (1.0.136 version). The analyzed geographic incidence rates of NHL include locations of the central and east provinces Orkhon, Uvurhangay, Khuvsgul, Ulaanbaatar and Dornod. The age-specific incidence and mortality rates were compared to those for all regions in Mongolia and those for the combined high mortality localities within the high-risk prefectures.



**Results:** Expression of Ki-67 protein was noted in 71.8% of the tumor cases. Average Ki-67 expression was associated with regions of high incidence.

**Conclusion:** We found that provinces with a high incidence and mortality from non-Hodgkin's disease were aggregated in the eastern-central parts of Mongolia, particularly in the areas along Ulaanbaatar capital city.

**Keywords:** [Non-Hodgkin's lymphoma \(NHL\)](#) – [Incidence](#) – [Mortality](#) – [Ki-67 index](#) – [Regions of Mongolia](#)[abstract](#).

## Introduction

Non-Hodgkin's lymphomas (NHLs) are a heterogeneous group of malignancies arising from lymphoid tissue. They vary in clinical and biological features as well as in their geographical distribution. North America and Europe display with a higher proportion of follicular and diffuse lymphoma, in contrast to Asia that reports a higher proportion of T-cell lymphoma [1,2]. The variation of NHL incidence in association with large geographic regions is well known.

Mongolia is one of the [most sparsely populated](#) sovereign countries in the world. It covers a population of around 3 million people, and has been progressively urbanized with even over populated cities. Nomadic life style has been significantly changed during the last 25 years.

Recently Reena Nair et al. reported that the NHL incidence commonly ensues in crowded areas of urbanized places, and might be associated with social and economical factors [3]. For last 5-7 years, NHL incidence in Mongolia has increased remarkably. Local scientists and doctors noticed that national cancer registries and founded tissue - based diagnoses might be not sufficient to study analytical epidemiology of NHL. For example, until 2012, morphologic features were not available to correctly classify NHL and Hodgkin's diseases in Mongolia.

NHL are the most common subtypes in all tumors of the hematopoietic and lymphoid tissue. In addition, J. Tsengelmaa et al, reported that the diffuse large B-cell lymphoma was the most frequent subtype of NHL in reported incidences of national cancer registries [4]. According to the basic health statistics of Mongolia, 31 new cases of NHL were registered in 2011, increasing to 52 in 2012, and finally 35 in 2013. In addition, about 153 new cases of NHL are assumed to be diagnosed in years 2014-2016, including 64 new cases in 2016. Based on these data, an increase of malignant lymphoma cases can be calculated to 2%-3% per decade during the period of this study (2006 – 2016).

## Methods



*Epidemiology:* The reports of the Mongolian annual national health indicator, which display with the mortality statistics of malignant lymphomas, including NHL, were obtained from the Center of Health Development in Ulaanbaatar for the period 2006 to 2016. The population and social and economical status were taken from the “conception of development by regions” which have been administered by Mongolian government since 1999. It distinguished 21 provinces and 4 regions in Mongolia. We calculated the age-standardized incidence rates (ASR, per 100000 inhabitants) for 2014-2016 in relationship of the included regions.

*Specimens & Immunohistochemistry:* Tissue samples sent for routine diagnosis to both the National Pathological Center and the Etemo Clinic in Ulaanbaatar served for the IHC investigation. This group included 35 patients who have been admitted with NHL (C82–C85 and C96) to the Bone Marrow Transplantation Center and Hematology Clinic at the First State Central Hospital of Mongolia. It comprised 25 cases of lymph node tumors and 10 cases of extra nodular tumors.

The tissue samples were fixed in 10% buffered formalin and embedded in paraffin. In each case, hematoxylin and eosin-stained glass slides were viewed and diagnosed by pathologists. Antibodies and reagents originated from Dako (Copenhagen, Denmark), including the monoclonal mouse antibody against Ki-67(clone MIB1 diluted 1:50) served for IHC stains. The number and percentage of positively stained nuclei were evaluated at magnifications x 10 and x 20 (Olympus BX 51 light microscope, and the interactive DISKUS 4.0.8 software for computer-assisted image analysis).

*Statistical analysis* was performed using the STATA 11 package. The P value<0.05 was considered of significant probability in differences. The geographic illustration was conducted with the aid of the software R Studio [using by Mapping plots] (1.0.136 version).

## Results

### ***Cancer registration and histopathologic diagnosis of NHL in Mongolia***

The majority of cancer cases is registered in National Cancer Center. However, some types of cancer are registered in other medical centers too. For instance, the most of hematopoietic tumors are currently diagnosed and registered in the Center of Hematology and Bone Marrow Transplantation at the First State Central Hospital of Mongolia. In addition to these registries secondary level health care centers in 21 provinces independently register cancer patients and report cancer prevalence since 2008. Nationwide registered incidence rates of NHL and hematopoietic, microscopically confirmed diagnoses are only available in Mongolia since 2013.

### ***Descriptive epidemiology of NHL in Mongolia***



Substantial differences in the incidence and distribution of major NHL subtypes across geographic regions were observed in Mongolia. These seem to be related to host, racial, and environmental differences in particular regions. Developed countries report age-standardized incidence rates(per 100,000 per year) in men and women of 10.3 and 6.6, compared to 4.3 and 2.6, respectively in Mongolia [by Annual national health indicator 2015].

The prevalence of NHL in urban Mongolian areas was (196) and 1.2 times higher than in rural areas. The patients mean age in this study was calculated to 33.8 years (range 8-65 years). The sex ratio (men / women) was computed 2.1:1. The NHL incidence rates of in the Mongolian Central and East regions exceeded that of the West and Gobi (South) regions ( $p>0.05$ ; <Figure 1 and 2>).

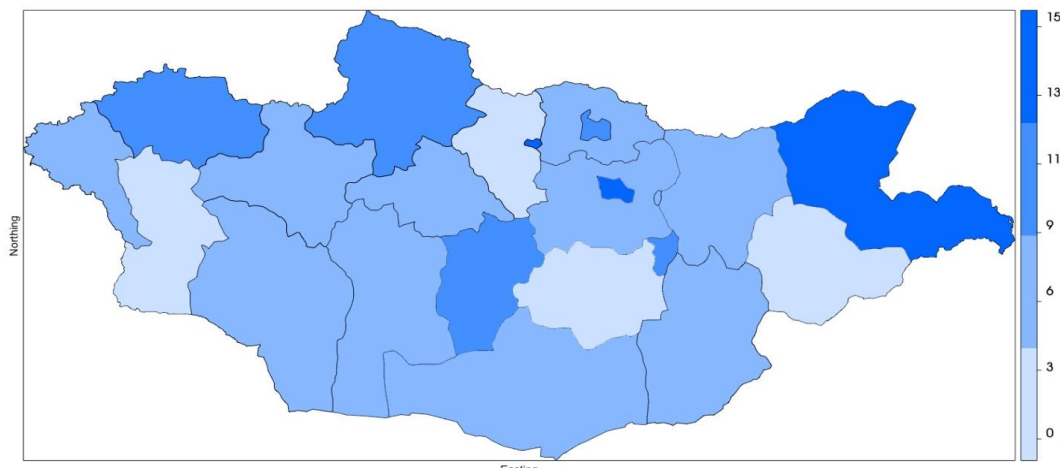


Figure 1: Map of Mongolia showing the geographical differences of age-standardized NHL incidence rates for years 2011-2016. The Data are compiled and visualized by R-Studio.

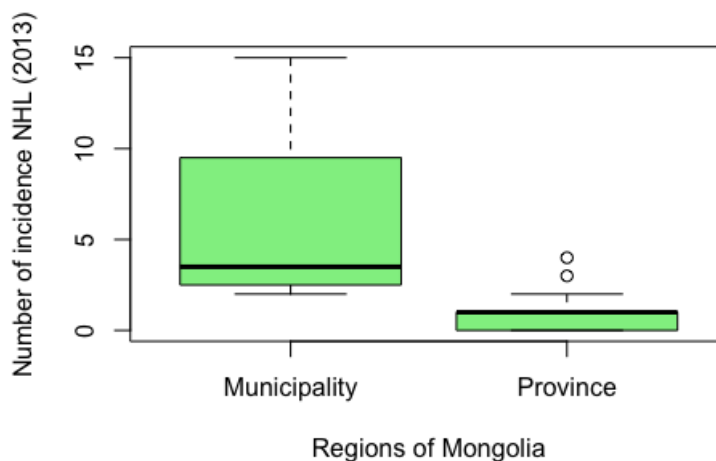


Figure 2: Annual frequency of NHL cases calculated for both Central and Rural areas in Mongolia ( $p<0.003$ ). Data compiled and visualized by R-Studio. (N=35).

#### Mortality and Surveillance analysis



The national NHL ASMR (age standardized mortality rate) in Mongolia was calculated 1.0 – 9.5 / 100,000 for the past 5 years (n=271), The ASMR of men exceeded that of women in each of the included provinces. <Table 1>

**Association of Ki-67 Expression with patient surveillance**

*Expression of Ki-67 antigen in NHL:* IHC reactions were performed for 35 NHL cases. Three cases had to be excluded from the study due to significant tissue damage. A positive (low, intermediate and high grade depending on cell cycles) Ki-67 reaction of the cell nuclei was observed in 23 cases (71.8%). <Figure 3 A, B>

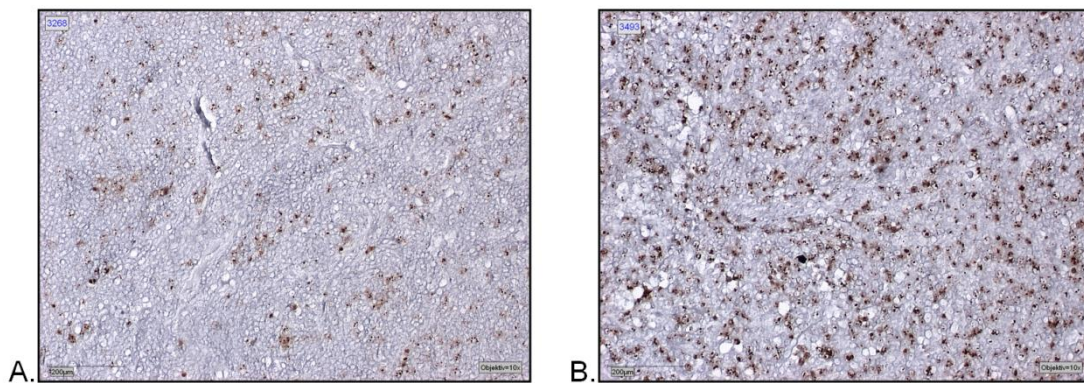


Figure 3 Immunohistochemical localization of Ki-67 expression in non-Hodgkin's lymphoma. A: With low proliferation rate; B: High proliferation rate. Magnification x10.

Regions/Provinces	Incidence					Mortality				
	Num. of Cases		of ASR (W)		M/F	Num. of Deaths		of ASMR		M/F
	M	F	M	F		M	F	M	F	
<b>Central Region</b>										
Ulaanbaatar	81	33	9.5	6.2	1.5	74	41	3.4	2.7	1.2
Uvurhangay	11	6	6.4	3.1	2.0	7	1	2.1	1.0	2.1
Orhon	14	10	7.0	6.6	1.0	10	2	3.0	1.4	2.1



Hövsgöl	8	4	8.0	4.3	1.8	7	1	0.5	0.4	1.2
Selenge	2	1	1.1	1.3	1.0	5	2	0.4	0.2	2.0
Töv	3	2	5.9	3.4	1.7	3	3	2.1	2.0	1.0
Arhangay	0	3	2.4	5.0	1.0	1	1	1.4	1.0	1.4
Darhan-Uul	7	4	6.9	6.3	1.0	8	6	3.1	1.9	1.6
Bayanhongor	3	2	3.7	4.0	1.0	4	2	1.1	1.1	1.0
Bulgan	2	0	2.9	1.2	2.4	0	0	1.0	1.0	1.0
<b><u>West Region</u></b>										
Uvs	7	3	9.5	7.0	1.3	7	2	2.8	1.9	1.5
Hovd	3	2	7.3	6.1	1.1	4	0	2.3	1.6	1.4
Bayan-Ölgiy	3	2	2.4	2.0	1.2	3	1	1.2	0.9	1.3
Dzavhan	3	1	2.9	1.8	1.6	3	0	2.2	1.2	1.8
Govi-Altay	2	2	1.0	4.3	1.0	0	1	0.8	0.3	2.6
<b><u>East Region</u></b>										
Dornod	17	7	10.2	6.9	1.4	11	9	3.4	2.9	1.1
Hentiy	1	2	2.0	2.4	1.0	0	0	1.0	1.0	1.0
Sühbaatar	2	1	3.1	2.2	1.4	1	0	0.8	0.5	1.6
<b><u>Gobi Region</u></b>										
Ömnögovi	2	1	4.4	2.8	1.5	1	0	1.1	1.0	1.1
Dornogovi	3	0	7.0	2.8	2.5	4	1	1.6	1.1	1.4
Dundgovi	2	1	6.0	4.8	1.2	0	1	2.7	2.1	1.2
Govisumber	6	2	10.2	8.7	1.1	2	2	3.0	2.2	1.3

Table 1: Age standardized incidence and mortality rates from NHL in Mongolia by regions.

**Regions** **Percentage of positive cells**



	Num. of Cases		0	$\leq 50.0$	$>50.0$	Mean (SD) Incidence of positive cases
Central	19	4	11	4	1.82 (1.05)	
West	4	2	2	0	0.85 (0.45)	
East	7	2	3	2	1.76 (1.45)	
South	2	1	1	0	1.3 (0.0)	

Table 2: Expression of Ki-67 (n=32).

Relationship between Ki-67 antigen expression and geographical distribution data in our studies a hypothetical relationship was tested between Ki-67 expression and geographical variables in the patients. The results of Ki-67 expression are shown in <Table 2>. The highest percentages of Ki-67 positive cells (range 57-73%) were observed in regions with high-grade NHL incidence. The lowest values were observed in patients in west-southern regions. Overall, the results in the cases of discovered in central-eastern regions were higher than those in west-southern area; this difference was significant ( $p < 0.02$ ).

## Discussion

Regions and towns with a high mortality and incidence of NHL aggregate in the central-eastern parts of Mongolia, particularly in areas of urbanized major cities. This finding confirms the recent report of Nair et al. that NHL incidences are higher in urban areas compared to rural regions [1, 3]. Lifestyle specificities, industries, demographic, social and economical factors might affect the epidemiology of this disease.

A worldwide relatively high incidence rate of NHL and other malignant lymphomas in urban environments or big cities was reported in another study based on biopsy specimens [5]. These findings may suggest a common etiologic agent for lymph reticular malignancies preferable in urban areas. In Mongolia the registration of NHL cases and consecutively the calculation of its prevalence was still insufficient, especially tissue – based examinations that are mandatory to classify the different diseases were missing. The analysis of our limited, tissue - based cohort confirms already the reports of studies, which are based upon tumor registries in developing countries [6].





In this study we found a relationship between the expression of the Ki-67 protein (proliferation rate), and geographically high incidence regions, the mortality rate, the patients' survival and quality of life. Our results are in agreement with data reported by Broyde et al., who found a similar expression of Ki67 protein in NHL and its association to the patients' survival [7].

Furthermore, a study reported that the expression of ALK and Ki-67 in combination with the clinical stage is a significant prognostic factor for NHL patients [3]. However, the clinical stage only could be confirmed as independent prognostic marker in a multivariate analysis [8]. Thus, it cannot be excluded that different prognostic factors may play a role in different age or sex cohorts, or in different national populations. A larger scale and preferably multi-institutional study is needed to confirm the prognostic role of geographical or ethnic differences [8].

In agreement with these findings, tissue - based diagnosis and reliable nationwide registration of hematopoietic tumors such as lymphoma is crucial to confirm and to finally compare the results with those of other international studies.

## Conclusion

We found that NHL incidence rates vary in different geographic regions of Mongolia. They might serve for investigate in etiology, pathogenesis, and environmental factors of NHL. Therefore, a reliable nationwide registration of NHL in order to more precisely define its prevalence should be elaborated in national medical health services of developing countries such as Mongolia.

A high rate of Ki-67 expression could be demonstrated in most NHL cases. The concrete relationship was established between the expression of Ki-67 antigen and their geographical variables in the NHL group. In fact the determination of Ki-67 expression in non-Hodgkin's lymphoma cells seems to be an informative and simple tool for assessing disease etiologic factors and its mechanisms. The evaluation of proliferative activity using the Ki-67 index allows a group of NHL patients with a predicted survival time in varies of regions to be distinguished.

## Disclosure Statement

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## Conflict of Interest

Authors declare no conflict of interest.





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