



# Expression of Neuron-Specific Enolase and Other Neuroendocrine Markers is Correlated with Prognosis and Response to Therapy in Non-Hodgkin Lymphoma

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## Abstract

**Objective** Non-Hodgkin lymphoma (NHL) is a common hematological malignancy. There is very little known about the expression of neuroendocrine immunohistochemical markers and their clinical significance in NHL due to the paucity of studies. Our objective was to study the expression of neuroendocrine immunohistochemical markers in NHL and correlate with clinical parameters.

**Materials and Methods** All cases diagnosed as NHL on morphology and immunohistochemistry (World Health Organization, 2016 classification) were included in the study. Immunohistochemistry for neuron-specific enolase (NSE), synaptophysin, and chromogranin A was performed. The results were correlated with clinical parameters and response to chemotherapy.

**Results** A total of 66 cases were included in the study with a male-to-female ratio of 3.1:1. The most frequent subtypes observed were diffuse large B-cell lymphoma not otherwise specified and follicular lymphoma. Among the neuroendocrine markers, positivity was observed only for NSE, whereas the other markers were uniformly negative. It was positive in both B- and T-cell lymphomas and in many different subtypes. No relation with the age and sex of the patients was observed. However, NSE-positive cases, more frequently, presented in the advanced stage as compared with NSE negative (61 vs. 38%). All NSE-positive cases showed remission with chemotherapy.

**Conclusion** Among the neuroendocrine immunohistochemical markers, positivity was observed only for NSE. This isolated positivity suggests cross-binding of NSE antibodies with some other isoenzyme of NSE. NSE positivity was associated with higher stage and better response to therapy. Despite this apparent paradox, it is recommended that NSE should be part of routine immunohistochemical panel for NHL.

## Keywords

- ▶ Immunohistochemical markers
- ▶ Lymphoma
- ▶ Neuroendocrine
- ▶ Neuron-specific enolase
- ▶ Non-Hodgkin

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## Introduction

Non-Hodgkin lymphoma (NHL) is a common hematological malignancy. As per Globocan 2012, the age-adjusted incidence rates for NHL in men and women in India are 2.9/100,000 and 1.5/100,000, respectively. These are about one-fourth of the incidence rates reported from Western Europe or North America. Within India, there is wide variation and the incidence is several-fold higher in urban areas compared with rural areas.<sup>1</sup> It is believed that with increasingly urban lifestyles and economic progress the incidence of NHL is likely to increase in India.<sup>1</sup>

Neuroendocrine immunohistochemical markers are primarily used to confirm the presence and diagnosis of neuroendocrine tumors. Ultrastructurally, the expression of these markers correlates with the presence of dense core granules in these tumors.<sup>2</sup> The markers that are most widely used in diagnostic practice world over are synaptophysin and chromogranin A.<sup>1,3-5</sup> In addition to these two, the third immunohistochemical marker which is often used is neuron-specific enolase (NSE).

Synaptophysin is also known as major synaptic vesicle protein p38 (SY38). It is normally present in the presynaptic vesicles of neurons and in similar vesicles of the adrenal medulla.<sup>5</sup> The gene for this protein (*SYP* gene) is located on X chromosome (Xp11.23-p11.22).<sup>4</sup> The protein has four transmembrane domains weighing 38kDa. The normal function of synaptophysin is not yet clear, but it is believed to interact with another protein synaptobrevin.<sup>6</sup>

Chromogranin A is also known as parathyroid secretory protein 1.<sup>11</sup> It is a member of the granin family of neuroendocrine secretory proteins.<sup>7</sup> These proteins are located in secretory vesicles of neurons and endocrine cells such as islet  $\beta$ -cell secretory granules in the pancreas. It is encoded by the *CHGA* gene which is located on chromosome 14q32.12.<sup>8</sup> The normal function of chromogranin A is to act as a precursor to the formation of various peptides with important function such as vasostatin-1, vasostatin-2, pancreastatin, catestatin, and parastatin.<sup>9</sup>

NSE is also known as gamma enolase or enolase 2 (ENO2). Biochemically, it is a phosphopyruvate hydratase which is an enzyme involved in the glycolytic pathway and is found mainly in neural and neuroendocrine cells. It plays a role in interconverting 2-phosphoglycerate and phosphoenolpyruvate.<sup>10</sup> It is encoded by the ENO2 gene located on chromosome 12 (12p13.31) on which there are three genetic loci that have been named as  $\alpha$ ,  $\beta$ , and  $\gamma$ .<sup>12</sup> NSE is gamma-gamma enolase.

The role of these immunohistochemical markers in the diagnosis of neuroendocrine tumors is well established.<sup>4</sup> However, there is very little known about the expression of these immunohistochemical markers in NHL because of the paucity of the literature. The idea for the present study came from a serendipitous observation when one of the neuroendocrine immunohistochemical markers (NSE) was put inadvertently on a case of NHL by the laboratory technologist and it came out to be positive.

In the earliest study on this subject, Nemeth et al<sup>13</sup> studied the immunohistochemical expression of NSE in 23

cases of malignant lymphoma. They concluded that NSE might be an inconstant marker of malignant with no apparent correlation between reactivity and morphology or phenotype. In another study, Massarelli et al<sup>14</sup> found NSE expression only in CD30-positive NHL. However, in none of these studies, the expression of neuroendocrine markers has been correlated with clinical parameters and response to therapy. Also, to the best of our knowledge, there is no study from India on this subject. Thus, the present study was undertaken to explore whether the expression of neuroendocrine markers in NHL may be of some clinical utility.

## Materials and Methods

The study was performed in the Department of Pathology and Department of Medical Oncology, of a tertiary care hospital from January 2016 to January 2019. All cases diagnosed as NHL in this period were included in the study. The study was duly approved by the institutional review board. Any patient who had received chemotherapy previously was excluded from the study. The diagnosis was made by morphological examination of hematoxylin and eosin (H&E)-stained sections and an extensive immunohistochemical panel. These cases were classified using the revised World Health Organization (WHO) classification of lymphoid neoplasms (2016).

Paraffin-embedded tissue blocks were used for cutting sections on poly L lysine-coated slides. Immunohistochemistry was performed using the avidin-biotin-peroxidase complex method. It was performed using NSE (Clone—MIG N3, Biogenex United States), synaptophysin (Clone—SNP 88, Biogenex, United States), and chromogranin (Clone—LK2H10, Biogenex, United States) primary antibodies. In brief, sections measuring 3 to 4  $\mu$ m thick were cut, deparaffinized with xylene, and brought to water through graded levels of alcohol. Endogenous peroxidase activity was blocked by treating the slides with hydrogen peroxide for 30 minutes at room temperature. Antigen retrieval was done using the pressure cooker method by immersing the slides in a citrate buffer. Then, the slides were incubated overnight with the primary antibody (pre-diluted) at 4°C in a humidified chamber. The following day secondary antibody was added. The sections were then incubated with di-aminobenzidine chromogen for the visualization of the peroxidase reaction. After being washed in water, the sections were counter-stained with hematoxylin, dehydrated in alcohol, cleared in xylene, and mounted.

The expression of NSE, synaptophysin, and chromogranin was then observed among all the cases of NHL as well as within the various subtypes of NHL. In all these cases, positivity was taken to be the presence of granular cytoplasmic staining.

Clinical parameters including clinical staging were obtained in all the cases and correlated with results of immunohistochemical staining. The patients were followed-up and response to therapy was noted. The status of bone marrow involvement was also obtained and correlated with results of immunohistochemistry for neuroendocrine markers.

Cases of benign reactive and inflammatory conditions of lymph node were used as control ( $n = 15$ ).

Statistical analysis was performed using student *t*-test and chi-square test on SPSS software ver. 21.0 (IBM). *p*-Value less than 0.05 was taken as significant.

## Results

The study was performed in the Department of Pathology and Department of Medical Oncology, of a tertiary care hospital from January 2016 to January 2019. During this period, a total of 66 cases were diagnosed as NHL and were included in the study.

There were 50 males and 16 females with a male-to-female ratio being 3.1:1. The overall age range was 14 to 82 years with median age being 46 years. The age range among males was 14 to 82 years with median age being 43 years and mean  $\pm$  standard deviation (S.D.) being  $43.6 \pm 21.4$  years. The age range among females was 32 to 79 years with median age and mean  $\pm$  S.D. being 47 years and  $49.5 \pm 17.9$  years, respectively. The difference in age between the two groups was not found to be statistically significant ( $p$ -value = 0.1).

As mentioned above, all these cases were categorized according to the revised WHO classification of lymphoid malignancies (2016). The distribution of male and female cases according to this classification schema is shown in ►Table 1. The number of cases of each subtype and their percentage are also shown in ►Table 1. As per the broad categories, there were 52 (79%) cases of mature-B-cell group, 13 (19.5%) of mature-T-cell group, and one (1.5%) of precursor-T-cell group. As can be seen in the table in both the groups, the most frequent subtype was diffuse large B-cell lymphoma (DLBCL) not otherwise specified (NOS) (►Fig. 1a) constituting 40 and 38% of all the cases among males and females, respectively. The second most common type was follicular lymphoma (►Fig. 2a) in both the groups (males 26% and females 19%). Among the females, the second spot was shared with anaplastic large cell lymphoma (ALCL) (►Fig. 3a) which also comprised 19% of cases. Other notable subtypes were small lymphocytic lymphoma and chronic lymphocytic leukemia (SLL-CLL) (►Fig. 4a) and peripheral T-cell lymphoma (►Fig. 5a).

The results of neuroendocrine immunohistochemical markers are shown in ►Figs. 1b–5b,6 and ►Table 1. As can be observed among the neuroendocrine immunohistochemical markers, only NSE showed positivity whereas chromogranin and synaptophysin were consistently negative in all the cases. The subtypes of NHL which showed positivity in both the sexes included all except mucosa-associated lymphoid tissue (MALT) lymphoma, T lymphoblastic lymphoma, and T-cell-rich large B-cell lymphoma. Most frequently, positivity was seen in ALCL (males 75% and females 67%). This was followed by peripheral T-cell lymphoma NOS (males 67% and females 100%) and DLBCL NOS (males 35% and females 50%).

NSE expression was correlated with the age of the patient, sex, tumor stage, and response to therapy, and results are shown in ►Table 2. For the purpose of this analysis, all the

cases were divided into four age groups less than 20, 20 to 40, 40 to 60, and greater than 60 years. As can be seen in the table, the NSE positivity was seen in all four age groups. The frequency of positivity ranged from 35% in 20 to 40 years' group to 45% in 40 to 60 age group. However, the difference between four age groups was statistically not significant ( $p$ -value = 0.09).

On correlating with sex, it was seen that among males out of a total 50 cases 20 showed positivity (40%), while in the females out of 16, seven showed positivity (44%). However, the difference between the two groups was statistically not significant ( $p$ -value = 0.07). However, when correlation was done with the tumor stage, it was seen that among the NSE-positive cases ( $n = 27$ ), 17 cases (61%) presented in the advanced stage (stage III/IV). On the contrary, in the NSE negative ( $n = 39$ ) group, only 15 cases (38%) presented in the advanced stage (stage III/IV). This difference was statistically significant ( $p$ -value = 0.001).

An attempt was made to correlate NSE positivity with other clinical variables like the extent of lymphadenopathy, hepatomegaly, and splenomegaly. However, no definite correlation was seen. Out of all the cases in which bone marrow status was known, only one case showed NSE positivity. In this case, the bone marrow was not involved. However, due to small number, statistical analysis could not be done.

There were three cases of extranodal lymphoma in the study. Among the males, there were one case each of DLBCL NOS of sinonasal region and a gastric MALT lymphoma. In the females, there was a case of peripheral T-cell lymphoma (PTCL) NOS of liver. Interestingly, all these three cases were negative for NSE.

The majority of these cases were given the standard CHOP chemotherapy. In cases of B-cell lymphomas which were positive for CD20, rituximab was added (R-CHOP). Patients of PTCL NOS and ALCL were given higher dose CHOP therapy. Patients were followed up for up to a period of 3 years; however, 36 cases were lost to follow-up. In the 30 cases where extended follow-up was available, five were NSE positive. All five, that is, 100% of them achieved (complete [ $n = 3$ ], partial [ $n = 2$ ]) remission. However, amongst the rest 25 NSE-negative cases, seven cases (28%) had persistence of residual disease after chemotherapy and 18 (72%) achieved remission (►Table 2). The difference between the two was statistically significant ( $p$ -value = 0.0001).

## Discussion

The present study is the first study from India to look at the expression of neuroendocrine immunohistochemical markers in NHL from India. A total of 66 diagnosed cases of NHL were included in the study. The study found a marked preponderance of males in the study population with a male-to-female ratio of 3.1:1. This is even higher than the male-to-female ratio reported from this location (Delhi) in the national cancer registry which is 2.2:1.<sup>13</sup> It is also in stark contrast to the data on the male-to-female ratio from Asia, Europe, and North America which is 1.6, 1.1, and 1.2, respectively.<sup>13</sup> The reason for a higher male-to-female ratio

**Table 1** Distribution of various diagnostic categories among male and female patients along with results of staining for neuroendocrine immunohistochemical markers

Male patients								
S.No.	Diagnosis	No. (%)	NSE		Synaptophysin		Chromogranin	
			Positive no. (%)	Negative no. (%)	Positive no. (%)	Negative no. (%)	Positive no. (%)	Negative no. (%)
<i>Mature B-cell neoplasms</i>								
1.	Diffuse large B-cell lymphoma (DLBCL), NOS	20 (40%)	7 (35%)	13 (65%)	Nil	13 (100%)	Nil	13 (100%)
2.	CLL/SLL	3 (6%)	1 (33%)	2 (66%)	Nil	3 (100%)	Nil	3 (100%)
3.	Follicular lymphoma	13 (26%)	4 (31%)	9 (69%)	Nil	13 (100%)	Nil	13 (100%)
4.	Mantle cell lymphoma	3 (6%)	2 (67%)	1 (33%)	Nil	3 (100%)	Nil	3 (100%)
5.	MALT lymphoma, gastric	1 (2%)	Nil	1 (100%)	Nil	1 (100%)	Nil	1 (100%)
<i>Mature T-cell neoplasms</i>								
6.	Anaplastic large cell lymphoma	4 (8%)	3 (75%)	1 (25%)	Nil	4 (100%)	Nil	4 (100%)
7.	Peripheral T-cell lymphoma NOS	5 (10%)	3 (60%)	2 (40%)	Nil	5 (100%)	Nil	5 (100%)
<i>Precursor T-cell neoplasm</i>								
8.	T lymphoblastic lymphoma	1 (2%)	Nil	1 (100%)	Nil	1 (100%)	Nil	1 (100%)
Female patients								
<i>Mature B-cell neoplasms</i>								
1.	Diffuse large B-cell lymphoma (DLBCL), NOS	6 (38%)	3 (50%)	3 (50%)	Nil	6 (100%)	Nil	6 (100%)
2.	CLL/SLL	2 (12%)	1 (50%)	1 (50%)	Nil	1 (100%)	Nil	1 (100%)
3.	Follicular lymphoma	3 (19%)	Nil	3 (100%)	Nil	3 (100%)	Nil	3 (100%)
4.	T-cell rich large B-cell lymphoma	1 (6%)	Nil	1 (100%)	Nil	1 (100%)	Nil	1 (100%)
<i>Mature T-cell neoplasms</i>								
5.	Anaplastic large cell lymphoma	3 (19%)	2 (67%)	1 (33%)	Nil	3 (100%)	Nil	3 (100%)
6.	Peripheral T-cell lymphoma NOS	1 (6%)	1 (100%)	Nil	Nil	1 (100%)	Nil	1 (100%)

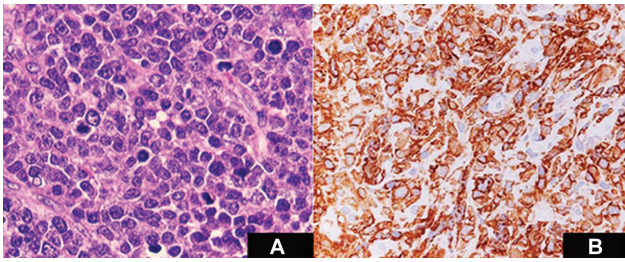
Abbreviations: CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; MALT, mucosa-associated lymphoid tissue; NOS, not otherwise specified.

in the present study could be due to a smaller sample size than previous studies.

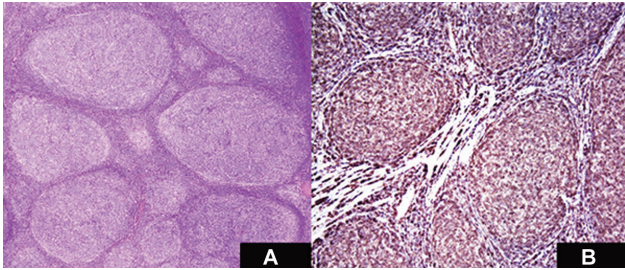
The overall median age was 46 years, while among males and females, it was 43 and 47 years, respectively. This is similar to the study performed by Sandhu et al<sup>15</sup> on a north Indian population in which they reported median age of 47 years. However, on comparison with western data, it is

observed that the median age is a decade less.<sup>15-18</sup> This clearly demonstrates that the clinical profile of NHL in India is different from western countries.

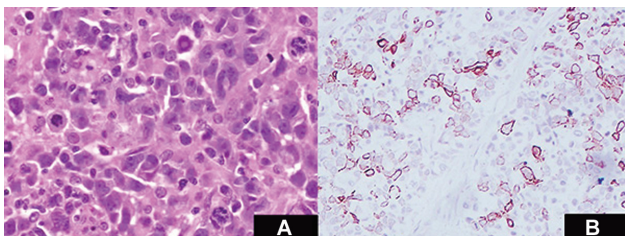
All the cases were diagnosed and categorized according to the revised WHO classification of lymphoid malignancies (2016). It was observed that overall mature-B-cell type was the most frequent broad category constituting 79% cases



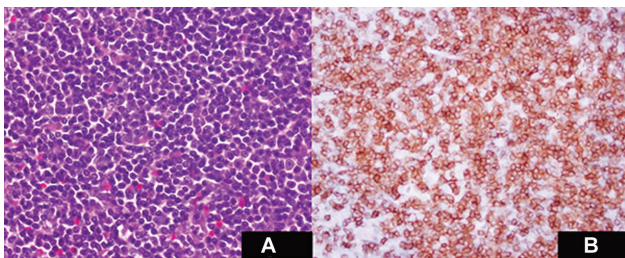
**Fig. 1** (A) DLBCL showing large pleomorphic cells with vesicular nuclei and prominent nucleoli. Abnormal mitoses are also seen (*arrow*) (H&E, ×400). (B) DLBCL showing cytoplasmic positivity for NSE (NSE, ×400).



**Fig. 2** (A) Follicular lymphoma showing closely spaced variably sized lymphoid follicles effacing normal architecture (H&E, ×40). (B) Follicular lymphoma showing positivity for NSE (NSE, ×100).

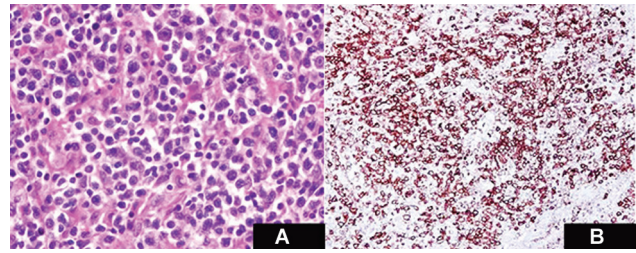


**Fig. 3** (A) ALCL showing large pleomorphic tumor cells with abundant cytoplasm. Mitoses are also seen (*arrow*) (H&E, ×400). (B) ALCL showing cytoplasmic positivity for NSE (NSE, ×200).



**Fig. 4** (A) SLL/CLL showing monomorphic population of small lymphocytes (H&E, ×400). (B) SLL/CLL showing positivity for NSE (NSE, ×400).

among both the sexes. This is in agreement with a previous study by Prakash et al<sup>19</sup> who have reported that B-cell NHL constitutes 80% of the cases of NHL. The most frequent subtype was DLBCL NOS which constituted 40 and 38% of all the cases amongst males and females, respectively. This is in agreement with previously reported data from large studies<sup>19–21</sup> performed in India which have consistently reported that DLBCL NOS is the most frequent subtype of



**Fig. 5** (A) PTCL showing variably sized tumor cells showing cytologic atypia (H&E, ×400). (B) PTCL showing positivity for NSE (NSE, ×200).

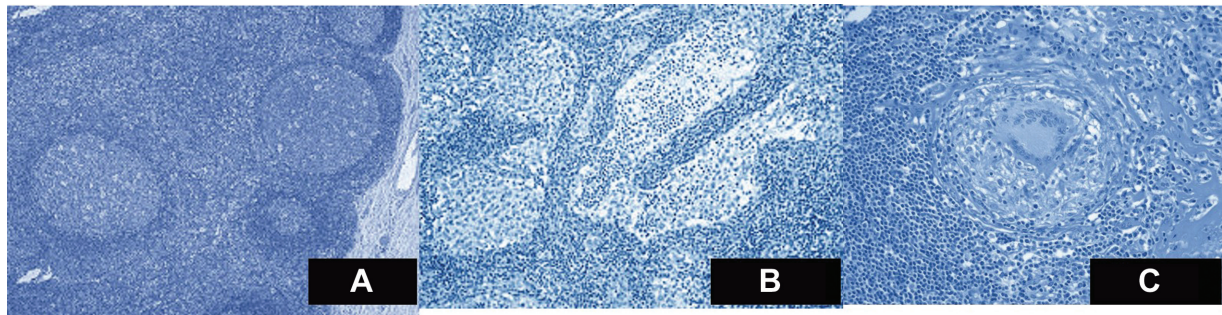
NHL in India. They have shown, in their studies, the prevalence ranging from 33.8 to 50.2% of all the cases.

The second most common type was follicular lymphoma in both the groups (males 26% and females 19%). This is also in perfect agreement with previous studies<sup>19–21</sup> which have also reported follicular lymphoma to be the second most common subtype. However, the overall prevalence in the present study of 24% is significantly higher than previous studies which vary from 10.5 to 13.1%. This may be due to the relatively smaller sample size in the present study. Among the females, a very high prevalence of ALCL was observed which comprised 19% of cases. This may again be due to the smaller sample size.

Among the neuroendocrine immunohistochemical markers, it was observed that only NSE showed positivity, whereas chromogranin and synaptophysin were consistently negative in all the cases. This raises an important question that whether the expression of NSE in NHL is a manifestation of aberrant neuroendocrine differentiation or it is due to the cross-binding of NSE antibody with another isoenzyme of NSE which is present in the increased concentration in these cases of NHL.

We would favor the second hypothesis. This is because of the following reasons. First, if it represented true neuroendocrine differentiation, some of the cases would have also shown positivity for synaptophysin and chromogranin. Second, unlike the other two, NSE is an enzyme whose positivity is not related to the presence of neurosecretory granules. On the contrary, synaptophysin is a membrane protein present in the synaptic vesicles.<sup>22</sup> These vesicles are distributed diffusely throughout the cytoplasm of neuroendocrine cells. Chromogranin is a constituent of the neurosecretory granules.<sup>23,24</sup> Thus, even a cell which does not contain neurosecretory granules may show positivity for NSE. Third, the most widely used clone MIG N3 for the detection not only binds to gamma-gamma enolase (NSE) but also detects the hybrid  $\alpha$ -gamma enolase. This is because the antibody is directed against the gamma enolase subunit which is present in both these isoenzymes of NSE.<sup>5</sup> This hybrid enolase besides neuronal and neuroendocrine cells is known to be present in a wide variety of cells including lymphocytes.<sup>25,26</sup> Thus, it is likely that the positivity with NSE seen in NHL is due to this binding of antibody with the hybrid enolase.

The positivity with NSE was seen in both B- and T-cell lymphomas. This is in contrast to the observations made by Massarelli et al<sup>14</sup> in their study who found positivity in only



**Fig. 6** (A) Follicular hyperplasia lymph node showing negative for NSE (NSE,  $\times 400$ ). (B) Sinus histiocytosis lymph node showing negative for NSE (H&E,  $\times 200$ ). (C) Granulomatous lymphadenitis showing negative for NSE (NSE,  $\times 400$ ).

CD 30-positive ALCL which is a T-cell lymphoma. However, it is in agreement with the findings of Nemeth et al<sup>13</sup> who found positivity for NSE in both B and T-cell lymphomas. Massarelli et al<sup>14</sup> also observed this phenomenon to be restricted to CD 30-positive ALCL. However, in the present study, we have observed this to be a more widespread phenomenon as many subtypes of NHL showed positivity including ALCL, DLBCL NOS, follicular lymphoma, mantle cell lymphoma, CLL/SLL, and peripheral T-cell lymphoma NOS. Among these, most frequently, positivity was seen in ALCL followed by peripheral T-cell lymphoma NOS and DLBCL NOS.

The NSE positivity in these cases was also correlated with the age of the patient. The cases were divided into four age groups less than 20, 20 to 40, 40 to 60, and greater than 60 years. It was observed that positivity for NSE was fairly uniformly distributed across all the age groups with the frequency of positivity ranging from 35% in 20 to 40 years' group to 45% in 40 to 60 age group. This suggests that the NSE positivity is not influenced by the age of the patient.

Similarly, when the correlation of NSE positivity was done with the sex of the patients, it was observed that in both the sexes positivity was seen. The frequency in males (40%) and females (44%) was also quite similar. Thus, it is likely that NSE positivity has no relationship with the sex of the patient. No correlation between NSE positivity and extent of lymphadenopathy, hepatomegaly, and splenomegaly was seen. As far as the relation with bone marrow status is concerned, it was observed in a case showing NSE positivity, bone marrow was not involved. However, due to the small number, no statistically significant conclusion could be drawn.

On correlating with the stage, it was seen that NSE-positive cases, more frequently, tend to present at an advanced stage compared with NSE-negative ones (61 vs. 38%). To explain this, we would like to hypothesize that this is because of the association of increased NSE expression with a higher metabolic state which might be correlating with greater cell turnover and higher tumor burden. However,

**Table 2** Correlation of NSE expression with age, sex, tumor stage, and response to therapy of patients

S. no.	Name of parameter	Total	Positive no. (%)	Negative no. (%)
	<i>Age group (y)</i>			
1.	< 20	10	4 (40%)	6 (60%)
2.	20–40	20	7 (35%)	13 (65%)
3.	40–60	20	9 (45%)	11 (55%)
4.	> 60	16	7 (44%)	9 (56%)
	<i>Sex</i>			
1.	Males	50	20 (40%)	30 (60%)
2.	Females	16	7 (44%)	9 (56%)
	<i>Tumor stage</i>			
1.	Stage I	22	7 (26%)	15 (39%)
2.	Stage II	12	3 (13%)	9 (23%)
3.	Stage III	16	10 (35%)	6 (15%)
4.	Stage IV	16	7 (26%)	9 (23%)
	<i>Response to therapy</i>			
1.	Remission	23	5 (22%)	18 (78%)
2.	Persistent residual disease	7	Nil	7 (100%)

to prove this hypothesis, larger focused studies on this aspect are required.

An interesting observation in the study was that all three cases of extra-nodal lymphoma irrespective of the histological subtype or location were negative. Although this may be purely due to the smaller number, it will be interesting to observe this phenomenon in a larger series of cases of extra-nodal lymphoma.

NSE positivity was also correlated with the response to chemotherapy. Out of the 30 cases of which follow-ups were available, all cases which were NSE-positive achieved complete remission. However, among the NSE-negative cases, 28% had persistent residual disease. However, the numbers are small but it may suggest a better prognosis for NSE-positive cases of NHL. Wang et al<sup>27</sup> in their study also showed a significant difference in 5-year overall survival (OS) rate between the NSE-positive and NSE-negative groups (93 vs. 44%). However, in their study, they included only cases of DLBCL NOS treated by rituximab-based immunotherapy. Another study Wang et al<sup>27</sup> showed that among patients in a non-GCB subtype of DLBCL, there was a significant difference in the 5-year OS rate between the NSE-positive group and the NSE-negative group (28.3 vs. 81.6%).

In a recent study, Zhu et al<sup>35</sup> studied the role of NSE in the diagnosis of malignant pleural effusion. The authors observed that use of pleural NSE measurement in diagnosing malignant pleural effusion is limited and with low sensitivity. The clinical utility of the NSE assay should be combined with the results of other tumor markers examination and the detailed clinical information of patients. Further studies are needed to confirm the role of NSE in diagnosing malignant pleural effusion.

This apparently better response to chemotherapy in NSE-positive cases may be explained by the fact that NSE is an enzyme of glycolysis which is a key metabolic pathway. Increased NSE expression in these cases of NHL also represents a higher state of metabolism. It is a well-known fact that chemotherapy works better against metabolically active cells. Thus, it is likely that due to this fact the NSE-positive cases showed an excellent response to chemotherapy. Many authors have also evaluated serum NSE as a potential prognostic marker in many hematological malignancies such as lymphoblastic lymphoma,<sup>28,29</sup> pyothorax associated lymphoma,<sup>30</sup> lymphoid leukemia,<sup>31,32</sup> multiple myeloma,<sup>33</sup> and Hodgkin lymphoma.<sup>34</sup> However, due to the small number of these studies, definite guidelines cannot be framed, thereby pointing toward the need for many more studies in the future with a larger sample size.

## Conclusions

Thus, to conclude, NSE is expressed in many different types of NHL including both B and T- cell types. Most frequently, expression is seen in ALCL and DLBCL NOS. Its expression is associated with a better response to chemotherapy. There is no association between NSE expression with age and sex of patients. Due to its association with better prognosis, NSE may serve as a novel screening test. Therefore, future studies

with a larger sample size and longer follow-up are required to confirm the role of NSE in the management of NHL.

## Limitations

The limitations of the present study are the relatively smaller sample size and limited follow-up. The findings of the study are not conclusive enough to draw any definite conclusions. Thus, there is a need for future studies with a larger sample size and longer follow-up to confirm the role of NSE in the management of NHL.

### Authors' Contributions

Amit Kumar Yadav contributed to the concept and design of this work, analysis of the data, writing of the manuscript, review of the final version of the submitted manuscript, and publication approval. Somshanker Chowdhury contributed to concept and design of this work, collection of data, analysis of the data, writing of the manuscript, review of the final version of the submitted manuscript, and publication approval. S.P. Kataria contributed to concept and design of this work, clinical data collection, analysis of the data, writing of the manuscript, review of the final version of the submitted manuscript, and publication approval.

### Ethical Approval

The study has been conducted after taking institutional board approval.

### Funding

None.

### Conflict of Interest

None declared.

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