The Egyptian Journal of Hospital Medicine (October 2022) Vol. 89 (2), Page 7411- 7418

The Prognostic Significance of Baseline Serum Ferritin in Patients with Non-Hodgkin Lymphomas: A Prospective Single-Institution Study

Dina M. EI-Habashy[1], Eman A. Tawfik[1], Suzan A. Alhassanin[1], Amira F. Shehata[2], Amira I. Aldesoky*[1]
Departments of [1]Clinical Oncology and [2]Clinical Pathology, Menoufia University, Shebein El-Kom, Egypt
***Corresponding author:** Amira I. Aldesoky, **Mobile:** (+20) 01096909270, **E-Mail:** amira.i.ahmed73@gmail.com

ABSTRACT

Background: Hyperferritinemia has been linked to a worse prognosis in several types of cancer, and it is often seen in patients with malignancies.

Objectives: The goal of this research was to learn how baseline blood ferritin levels affect prognosis among cases who had non-Hodgkin lymphoma (NHL).

Patients and Methods: From May 2016 to April 2019, from the Menoufia University Clinical Oncology Department we gathered data prospectively. 77 patients with non-Hodgkin lymphoma had their clinicopathological features and serum ferritin levels evaluated. The Kaplan-Meier technique was used to calculate time to treatment failure (TTF) and overall survival (OS).

Results: High serum ferritin levels were significantly associated with advanced stage, poor performance status (PS), high/ high-intermediate international prognostic index (IPI), elevated lactate dehydrogenase (LDH) and elevated β2 microglobulin in NHL (P= 0.023, P= 0.002, P= 0.001, P <0.001and P <0.001 respectively) while hyperferritinemia was significantly associated with advanced stage and elevated β2 microglobulin in HL (P =0.036 and P =0.003 respectively). No statistically significant correlations were detected between baseline ferritin levels; and response to therapy, TTF and OS in NHL patients.

Conclusion: Malignant lymphoma patients whose baseline serum ferritin was high tended to have more advanced illness. However, no association between an increased ferritin level and the outcome of survival analysis was found.

Keywords: Serum Ferritin, Non- Hodgkin Lymphomas, Hyperferritinemia.

INTRODUCTION

Non-Hodgkin lymphoma (NHL) represents the most common hematological malignancy in the world and is considered a heterogenous group of malignancies of the immune system, with the majority of NHL are of B cell origin, and the remaining (about 10- 15%) arise from T cells or NK cells [1]. The International Prognostic Index (IPI) remains the most common prognostic tool used for high grade NHL; however, a great diversity is found among those who share the same IPI-risk category, an issue which might affect the prognostic value of IPI [2, 3]. Novel molecular biomarkers and gene expression profiles have been explored for possibility to be exploited as prognostic factors for patients with NHL, although, using these markers in practice is limited by being expensive, unavailable, and difficult to interpret [4].

Consequently, we are in constant need for an alternative prognostic marker, which meets the criteria of being continuously available, cheap, and easy to interpret [3]. The intracellular protein ferritin is primarily a surrogate marker for total iron reserves but also acts as an acute phase reactant and is shown to be raised in inflammatory diseases, infections, and cancers[5, 6].

Some reports have demonstrated that ferritin level is significantly elevated in hematological malignancies such as Hodgkin lymphoma and in solid tumors as well [6, 7]. Moreover, the rise in ferritin level was found to correlate to the aggressiveness of the tumor [8].

Recent data have revealed that high serum ferritin level had a detrimental outcome in patients with NHL [9]. In the same context, one study has studied, retrospectively, the effect of baseline serum ferritin on survival outcomes for patients of NHL after completion of their chemotherapy [10]. However, there are no previous reports, prospectively, examining the role of baseline serum ferritin in predicting survival outcomes for patients with high-grade NHL.

The study's goal is to identify the predictive value of baseline serum ferritin in patients with newly diagnosed high-grade NHL and to examine its relationship to survival outcomes and other prognostic indicators of NHL.

PATIENTS AND METHODS

Patients

This prospective study was held between May 2016 and April 2019 at Menoufia University Hospitals' Clinical Oncology and Clinical Pathology Departments. For this analysis, we included 77 people who had been diagnosed with NHL.

Patients who met both the study's inclusion and exclusion criteria took part. Pathological confirmation of a diagnosis of NHL was required for patient inclusion. All the patients had recently been diagnosed with NHL and underwent induction treatment. Prior to starting chemotherapy, participants' serum ferritin levels were assessed as a baseline. Patients diagnosed with low grade NHL and those who received chemotherapy for other reasons like, patients with recurrence or another primary tumor, were excluded from the study.

The included patients were classified into 2 groups according to their serum ferritin (Normal and high

Received: 15/07/2022
Accepted: 18/09/2022

ferritin-level groups). Clinical data about the patients as: age, gender, Ann Arbor stage, IPI score, extra-nodal involvement, and B symptoms, were, initially, collected. Patients enrolled in the study had previously been treated with chemotherapy such as CHOP (cyclophosphamide, Adriamycin, vincristine, and prednisolone) or RCHOP (rituximab with CHOP) +/- involved field radiotherapy (IFRT) for DLBCL, and CODOXM (methotrexate, doxorubicin, cyclophosphamide, as well as vincristine) alternating with IVAC.

The response to treatment was assessed according to international Harmonization Project Criteria for response criteria in lymphoma [11].

METHODS

Blood samples were obtained from the patients before start of treatment and basic biochemical parameters (CBC, liver, and kidney functions), inflammatory biomarkers (ESR, CRP), tumor markers (LDH, β2 microglobulin) and serum ferritin were measured. The serum ferritin concentration was determined using a tiny VIDAS immuno-analyzer, a portable automated immunoassay system based on the Enzyme Linked Fluorescent Assay (ELFA) method. Serum ferritin levels in the study were considered normal if they fell within the following ranges (male, 15-300 µg/l; female, 15-200 µg/l) [12]. Serum ferritin levels below 300 µg/l in males and below 200 µg/l in females were considered to be in the normal ferritin range, whereas levels above these ranges were considered to be in the high ferritin range.

A patient's Time to Treatment Failure (TTF) was calculated from the time of diagnosis until the occurrence of any treatment failure. From the time of diagnosis to the time of death from any cause or the end of follow-up, we determined the patients' overall survival (OS)[13].

Ethical consent:

All adult patients and every caregiver of child patient supplied signed informed consents, and the study was authorised by the Menoufia University Faculty of Medicine's Ethical Council. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

Statistical significance was assumed. Statistical analysis was performed using SPSS 22. Qualitative data were represented as frequencies and relative percentages. Chi square test ($\chi 2$) to calculate difference between two or more groups of qualitative variables. Quantitative data were expressed as mean ± SD (Standard deviation). The Kaplan-Meier method was used to estimate survival time, and the log-rank test was used to compare the results. Cox proportional hazard regression was used for univariate and multivariate analyses of TTF and OS. The multivariate Cox regression model only contained factors that were statistically significant in the univariate analysis. With a p-value lower than 0.05.

RESULTS

Table (1) provides a brief summary of patient characteristics. Patients enrolled ranged in age from 13 to 80, with a majority of females participating (51.9 percent). The vast majority of cases of NHL were diffuse large B cell lymphoma (90.9%). 26 patients were classified as having stage II disease according to the Ann Arbor staging system. According to the IPI risk classification system, 33.3 percent, fell into the low. The vast majority of patients received either standard CHOP-like treatment based on anthracyclines (n=30, 39%) or no chemotherapy at all (n=42, 54.5%).

Table (1): Patient characteristics

Characteristics	NHL
Median age (Range), years	55 (13-80)
Gender	
Male	37 (48.1%)
Female	40 (51.9%)
Comorbidities	
Hepatitis C virus +ve	30 (39%)
Hepatitis B virus +ve	0
PS	
0	38 (49.4%)
1	28 (36.4%)
2	11 (14.3%)
Stage	
I	14 (18.2%)
II	26 (33.8%)
III	14 (18.2%)
IV	23 (29.9%)
B symptoms	
Yes	26 (33.3%)
No	51 (66.2%)
Pathologic subtypes of NHL	
DLBCL	70 (90.9 %)
Burkitt lymphoma	4 (5.2 %)
High grade follicular	3 (3.9 %)
IPI for NHL:	
0	13 (16.9%)
1	22 (28.6%)
2	17 (22.1%)
3	14 (18.2%)
4	10 (13%)
5	1 (1.3%)
Extranodal disease	
Yes	49 (63.6%)
No	28 (36.36%)
Bulky disease	
Yes	15 (19.48%)
No	62 (80.5%)
Therapeutic regimens in NHL	
CHOP	42 (54.5%)
RCHOP	30 (39%)
CODOXM, IVAC	4 (5.2%)
High dose MTX and Arac	1 (1.3%)
Response to treatment	
CR	35 (44.9%)
PR	9 (11.5%)
SD	4 (5.1%)
PD	12 (15.4%)
Mean ferritin (ng/mL)	445.27 ± 108.21
Mean LDH (IU/L)	447.23 ± 101.64
Mean ESR (mm/1st h)	49.71 ± 12.34
Mean CRP (mg/L)	39.84 ± 9.36
Mean β2 microglobulin (mg/L)	3.51 ± 0.71

Ferritin levels in NHL patients

Ferritin median level was 180 (range 3.3-3520) and based on the reference range of serum ferritin, patients were classified into two groups; the first compromised 31 patients with high ferritin (HF) level, the second included 46 patients with normal ferritin (NF) level. Patients with HF were more likely to have a more advanced stage of disease, chronic liver disease, lower PS, high, high/intermediate IPI, elevated LDH, and elevated β2 microglobulin, than those in the NF group (Table 2).

Table (2): Association between ferritin levels and patient characteristics

	Normal ferritin (n= 46)	**High ferritin (n=31)**	**P value**
Age:			
≤ 60	27	16	0.74
> 60	17	14	
Stage			
I, II	30	10	**0.023***
III, IV	16	21	
Chronic liver disease:			
Yes	13	18	**0.009***
No	33	13	
PS			
0	30	8	**0.002***
1	13	15	
2	3	8	
IPI			
0, 1, 2	35	17	**0.001***
≥3	11	14	
Extranodal sites			
Yes	27	22	0.27
No	19	9	
B symptoms			
Yes	13	13	0.21
No	33	18	
Bulky disease			
Yes	7	8	0.25
No	39	23	
Response			
CR	24	11	0.15
No CR	16	15	
Survival			
Alive	31	16	0.051
Dead	9	13	
LDH (U/L)	362.04 ± 89.61	597.19 ± 148.31	**<0.001***
ESR (mm/hr)	43.13 ± 10.21	59.48 ± 13.51	0.067
CRP (mg/L)	37.11 ± 9.10	43.91 ± 10.12	0.234
β2 microglobulin	3.07 ± 0.72	4.16 ± 0.91	**<0.001***

Univariate and multivariate analyses

In univariate analysis, a significant association between high/intermediate risk IPI, elevated LDH and TTF was found, while, high risk IPI, elevated LDH above normal, advanced stage and high β2 microglobulin, were significantly associated with OS. Multivariate analysis showed high, high/ intermediate IPI was an independent prognostic factor for TTF (Figure 3a), while elevated LDH was significantly correlated with OS (Figure 3b) (Tables 3, 4).

Table (3): Univariate and multivariate analysis of prognostic factors for TTF

	Univariate analysis		Multivariate	
	95% CI	**P value**	**95% CI**	**P value**
Age > 60y	5.49 - 8.48	0.16		
IPI ≥ 3	4.96 – 7.04	**0.002***	0.49-1.13	**0.02***
LDH > ULN	8.58 – 11.12	**0.005***	0.42-2.75	0.85
PS ≥ 2	4.21 – 7.07	0.17		
Advanced Stage	6.13 - 8.82	0.10		
ESR > ULN	7.03 - 9.37	0.94		
B2 > ULN	6.25 - 8.87	0.22		
Ferritin > ULN	6.88 - 9.85	0.89		

Table (4): Univariate and multivariate analysis of prognostic factors for survival

	Univariate analysis		Multivariate	
	95% CI	**P value**	**95% CI**	**P value**
Age > 60y	10.56 - 17.04	0.30		
IPI ≥ 3	7.28 - 13.39	**0.007***	0.68- 1.81	0.66
LDH> ULN	7.74 - 11.64	**<0.001***	0.02- 0.61	**0.01***
PS ≥ 2	7.97 - 15.76	0.21		
Advanced Stage	9.18 - 14.00	**0.023***	0.36- 4.12	0.72
ESR > ULN	12.80 -16.88	0.86		
B2 > ULN	11.11 - 17.00	**0.04***	0.31- 2.41	0.78
Ferritin > ULN	9.04 - 14.24	0.051		

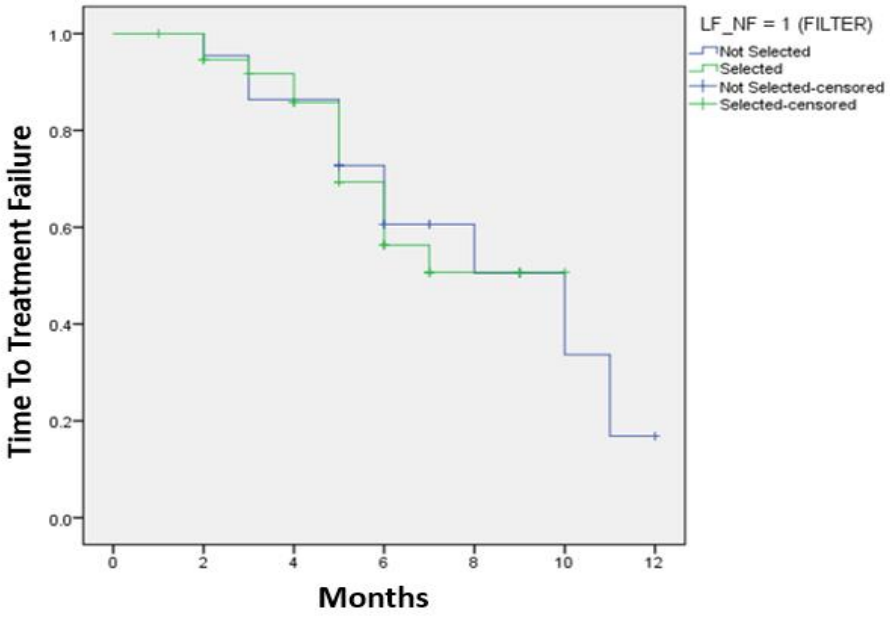


Figure (1): A non-significant correlation between TTF and elevated ferritin level in patients with NHL (P value = 0.89)

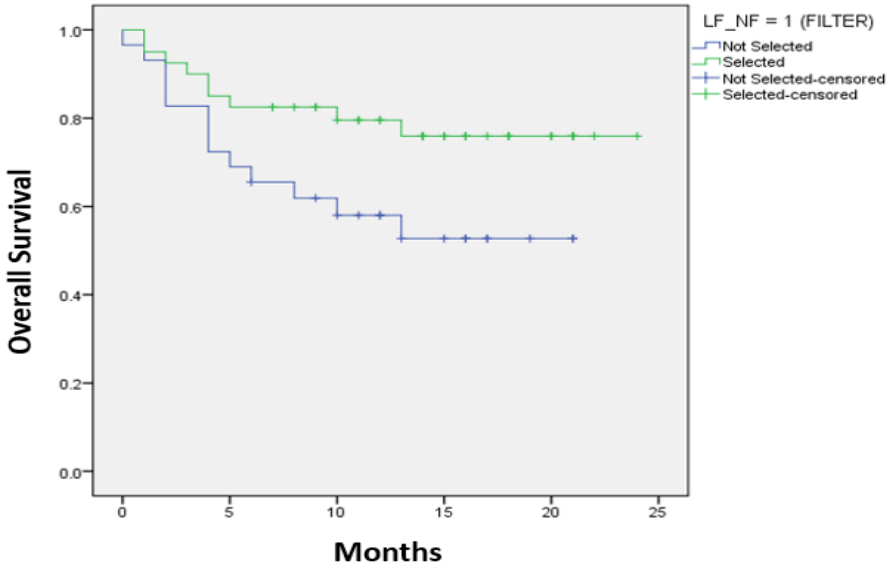
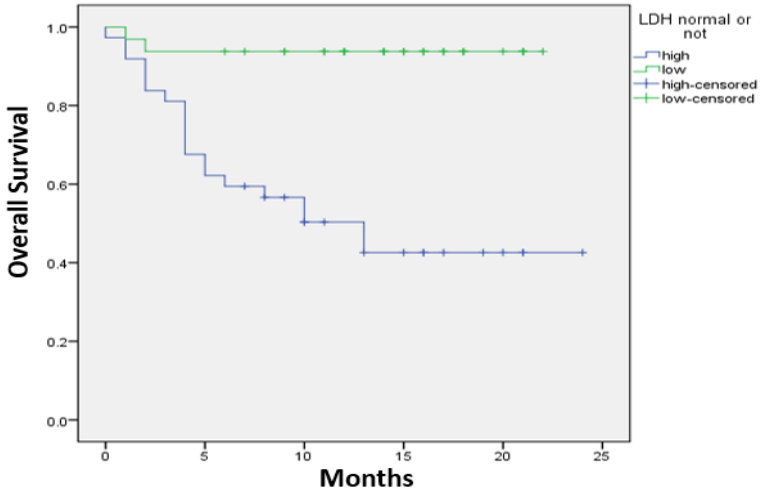
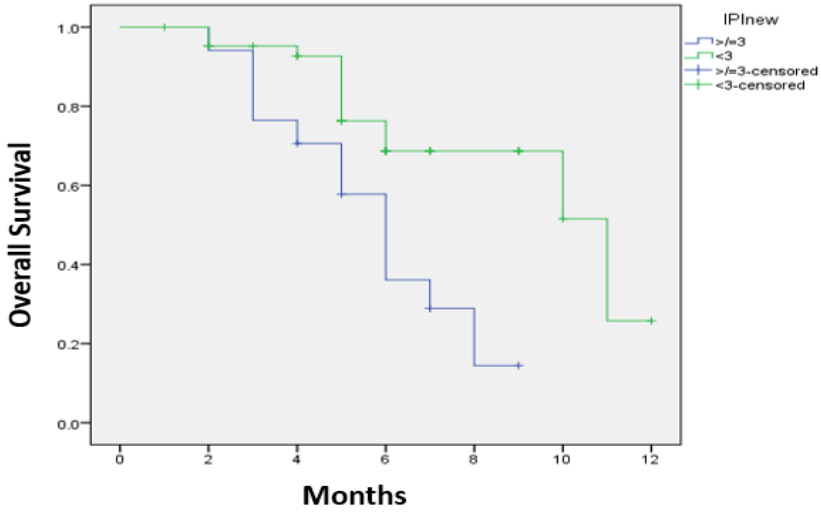


Figure (2): Shorter OS in patients with high ferritin level than in those with normal ferritin level. However, this difference was statistically not significant (P value = 0.051)



(a)



(b)

Figure (3): Overall survival (OS) according to LDH level (a) and time to treatment failure (TTF) according to IPI (b).

DISCUSSION

Of course, lymphoma encompasses a wide range of subtypes. Although the International Prognostic Index (IPI) is currently the gold standard for lymphoma prognostication, other promising prognostic markers have been investigated that may be able to identify high-risk patients, including novel molecular gene-expression profiling, genetic markers, and immunohistochemistry-based detection of prognostic biomarkers. Most of these identifiers, however, are prohibitively expensive and cryptic in their meaning. Thus, there is a continuing need for a low-cost, broadly accessible, and clearly interpretable prognostic predictor for patients with lymphoma [13].

In malignant lymphoma, serum ferritin levels were assumed to be connected to the disease's prognosis and response to treatment. No prospective studies examining the prognostic significance of baseline blood ferritin level in patients with malignant lymphoma were found in our search. Our prospective investigation showed that individuals with newly diagnosed malignant lymphoma had different outcomes depending on their baseline serum ferritin level.

We showed that elevated serum ferritin levels in NHL patients were substantially related to advanced disease stage, poor PS, high/high-intermediate IPI, elevated LDH, and elevated β2 microglobulins. This was consistent with previous research linking elevated serum ferritin levels to aggressive disease characteristics such poor prognosis, high LDH levels, and high/high-intermediate IPI [9, 14]. Considering the correlation between ferritin elevation and poor prognosis, it's possible that ferritin could serve as a biomarker in NHL.

In this study, the presence of chronic liver disease (caused mainly by HCV) correlated significantly with high serum ferritin levels in patients with NHL. This was in line with one study that reported that about 30-40% of patients with HCV had elevated serum iron and ferritin. Also, others have found that serum ferritin level was significantly elevated in response to chronic liver disease caused by HCV infection, non-alcoholic fatty liver disease and alcoholic liver disease [15].

In multivariate analysis, having a high/ high-intermediate IPI and elevated LDH above normal were found to be independent poor prognostic factor to TTF and OS respectively. These findings were in the same context with other reports at which IPI and LDH represented the widely accepted prognostic index to predict survival outcome in NHL [16, 17].

Unfortunately, no correlation between ferritin and either OS or TTF has been seen. Previous reports have revealed that patients with increased blood ferritin levels prior to therapy had a lower chance of surviving their NHL[10]. Peripheral T cell lymphoma patients with a raised serum ferritin level had a higher probability of having a poor prognosis, as revealed by these and other studies (PTCL) [14].

Although survival analysis in patients with NHL showed that OS was shorter in patients with high ferritin level than in patients with normal ferritin, this difference in OS between the 2 subgroups was statistically non- significant.

Our study has some drawbacks. The short period of follow up (median duration of follow up was 12 months) of the study group constitutes the most important one. This might have affected the correlation between the prognostic significance of the marker and the patients' outcome (whether therapeutic or survival outcome). A further limitation of this study is the small number of patients included in the study.

Heterogeneous treatment protocols represented another limitation. While some patients received combined immune chemotherapy with RCHOP, others have received CHOP only. In addition, intensive chemotherapy protocols were used in certain patients with Burkitt lymphoma and CNS lymphoma. This difference in treatment modalities might have affected the outcome of the patients.

In conclusion, we suggest that high ferritin levels might be associated with factors suggestive of aggressive disease like advanced stage, poor PS, high/ high-intermediate IPI, elevated LDH and elevated β2 microglobulin, however, the correlation between elevated ferritin levels and the outcome wasn't reached.

So, based on our study, we recommend that using baseline serum ferritin level as an inexpensive and widely available marker, with other prognostic factors, in patients with malignant lymphoma might be a useful prognostic and predictive biomarker. However, a longer duration of follow up is needed for proper correlation between ferritin level and survival outcome. We think, also, that carrying out more studies on larger numbers of patients and inclusion of patients receiving similar treatment methods may lead to more accurate results.

Financial support and sponsorship: Nil.
Conflict of interest: Nil.

REFERENCES

1. **Armitage J, Gascoyne R, Lunning M *et al.* (2017):** Non-Hodgkin lymphoma. The Lancet, 390: 298–310.
2. **Vaidya R, Witzig T (2014):** Prognostic factors for diffuse large B-cell lymphoma in the R(X)CHOP era. Annals of Oncology, 25: 2124–2133.
3. **Xin X, Liu Z, Meng F *et al.* (2015):** Analysis of prognostic factors in lymphoma patients with bone marrow involvement: A single center cohort study. Int J Clin Exp Med., 8: 9676–9683.
4. **Park J, Yoon D, Kim D *et al.* (2014):** The highest prognostic impact of LDH among International Prognostic Indices (IPIs): an explorative study of

Received: 15/07/2022
Accepted: 18/09/2022

five IPI factors among patients with DLBCL in the era of rituximab. Ann Hematol., 93: 1755–1764.

5. **Dignass A, Farrag K, Stein J (2018):** Limitations of serum ferritin in diagnosing iron deficiency in inflammatory conditions. Int J Chronic Dis., 1: 1–11.
6. **Alkhateeb A, Connor J (2013):** The significance of ferritin in cancer: Anti-oxidation, inflammation and tumorigenesis. Biochim Biophys Acta Rev Cancer, 1836: 245–254.
7. **Fernandez-Alvarez R, Gonzalez-Rodriguez A, Esther Gonzalez M *et al.* (2015):** Serum ferritin as prognostic marker in classical Hodgkin lymphoma treated with ABVD-based therapy. Leuk Lymphoma, 56: 3096–3102.
8. **Kalousová M, Krechler T, Jáchymová M *et al.* (2012):** Ferritin as an independent mortality predictor in patients with pancreas cancer. Results of a pilot study. Tumor Biology, 33:1695–1700.
9. **Yamazaki E, Tomita N, Koyama S *et al.* (2014):** Serum ferritin level is prognostic of patient outcome in extranodal NK/T cell lymphoma, nasal type. Medical Oncology, 31: 1–7.
10. **Yoh K, Lee H, Park L *et al.* (2014):** The prognostic significance of elevated levels of serum ferritin before chemotherapy in patients with non-Hodgkin lymphoma. Clin Lymphoma Myeloma Leuk., 14: 43–49.
11. **Cheson B, Fisher R, Barrington S *et al.* (2014):** Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: The Lugano Classification. J Clin Oncol., 32: 3059–3067.
12. **Bhatnagar N (2017):** Dacie and Lewis Practical Haematology (12th edition), By Bain B, Bates I, Laffan M. Elsevier, London. Pp. 652–652. https://onlinelibrary.wiley.com/doi/full/10.1111/bjh.14872
13. **Dührsen U, Müller S, Hertenstein B *et al.* (2018):** Positron emission tomography-guided therapy of aggressive non-Hodgkin lymphomas (PETAL): A multicenter, randomized phase III trial. J Clin Oncol., 36: 2024–2034.
14. **Koyama S, Fujisawa S, Watanabe R *et al.* (2017):** Serum ferritin level is a prognostic marker in patients with peripheral T-cell lymphoma. Int J Lab Hematol., 39: 112–117.
15. **Georgopoulou U, Dimitriadis A, Foka P *et al.* (2014):** Hepcidin and the iron enigma in HCV infection. Virulence, 5: 465-76.
16. **Ziepert M, Hasenclever D, Kuhnt E *et al.* (2010):** Standard international prognostic index remains a valid predictor of outcome for patients with aggressive CD20+ B-cell lymphoma in the rituximab era. J Clin Oncol., 28: 2373–2380.
17. **Yadav C, Ahmad A, D'Souza B *et al.* (2016):** Serum lactate dehydrogenase in non-Hodgkin's lymphoma: A prognostic indicator. Indian Journal of Clinical Biochemistry, 31: 240-42.