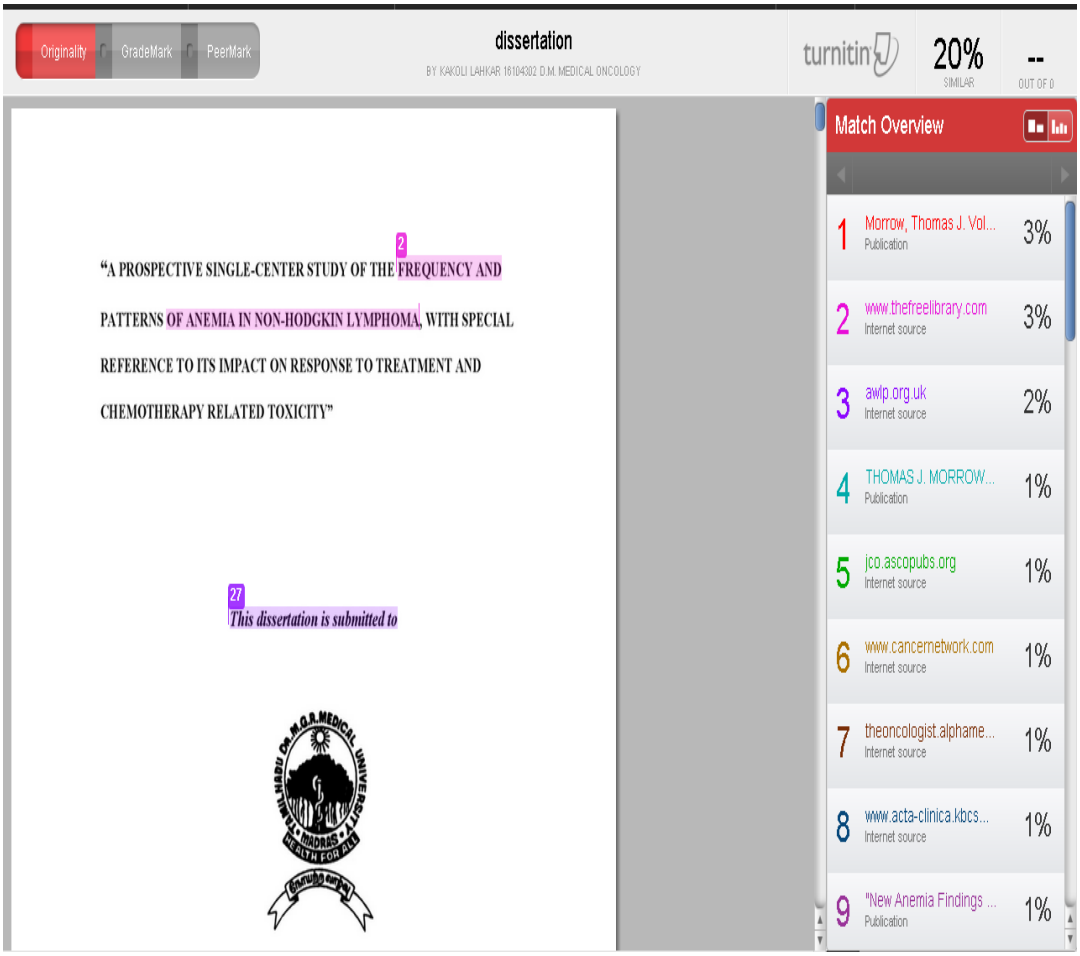
"A PROSPECTIVE SINGLE-CENTER STUDY OF THE FREQUENCY AND PATTERNS OF ANEMIA IN NON-HODGKIN LYMPHOMA, WITH SPECIAL REFERENCE TO ITS IMPACT ON RESPONSE TO TREATMENT AND CHEMOTHERAPY RELATED TOXICITY"

This dissertation is submitted to

The Tamilnadu Dr MGR Medical University, Chennai in partial fulfillment of the regulations for D.M. Medical Oncology(Branch VII) Degree Examination of August 2013

CANCER INSTITUTE (W.I.A)

Adyar, Chennai-600020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CERTIFICATE

This is to certify that this dissertation on "**A prospective single center study of the frequency and patterns of Anemia in Non-Hodgkin Lymphoma, with special reference to its impact on response to treatment and chemotherapy related toxicity"** is a bonafide work done by the candidate with **Reg.No. 16104302** in the Department of Medical Oncology, in partial fulfillment of the regulations for D.M Medical Oncology(Branch VII), Batch 2010-2013, College of Oncological sciences, Adyar, Chennai,, under my overall supervision and guidance, to my satisfaction.

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CONTENTS

Introduction :

Anemia is defined as a hemoglobin (Hb) level less than 13gram/deciliter (g/dl) in men and less than 12g/dl in women by the World Health Organization(WHO).[1] Anemia affects a quarter of global population including 293 million children and 468 million non-pregnant women and the incidence is much higher in India[2]. The critical elements of erythropoiesis include erythropoietin (EPO), iron availability, proliferative capacity of the bone marrow and effective maturation of precursor cells in the marrow. These factors are involved in the causation of different types of anemia.[3].

Correct assessment of the cause and type of anemia is essential for its treatment. The symptoms of anemia include fatigue, decreased work tolerance, shortness of breath, palpitations or other signs of cardiopulmonary adjustments to anemia [4,5].

The evaluation of anemia includes a thorough history and physical examination followed by various investigations. The initial evaluation includes an assessment of whether anemia is associated with other hematological abnormalities. Further evaluation of anemia consists of assessment of reticulocyte response, peripheral smear study, a determination of iron indices, a bone marrow aspiration to study cell morphology and iron stores.

Anemia is a common finding in patients diagnosed with cancer. Also, in such patients, the magnitude of anemia may be masked by a contraction of

plasma volume [6]. Anemia is widely prevalent in lymphoproliferative diseases even in patients without involvement of bone marrow and prior to initiation of chemotherapy [7, 8, 9].

The prognostic significance of anemia in Hodgkin lymphoma(HL) is well known and is part of the International Prognostic Scoring System (IPSS) in HL[10] . The impact of anemia in prognosis of lymphoid malignancy is also evident in follicular lymphoma and is part of Follicular lymphoma International Prognostic Index (FLIPI)[11]. However, the prognostic significance of anemia in other subtypes of non-Hodgkin lymphoma (NHL) is not known and few studies have looked into this [7]

The present study titled "A prospective single-center study of the frequency and patterns of anemia in non-Hodgkin Lymphoma, with special reference to its impact on response to treatment and chemotherapy related toxicity" is a single centre study performed in a tertiary cancer care centre in south India. This study is conducted to assess the patterns of different types of anemia and its impact on attainment of response and chemotherapy induced toxicity in patients with NHL in our population.

The Aims and Objectives of the study are as follows:

- To study the frequency of anemia in newly diagnosed patients with NHL
- To analyze the patterns and causes of anemia in patients with NHL
- To study the association of anemia with age , sex, stage and different subtypes of NHL
- To study the association of anemia with attainment of Complete response(CR) and Partial response(PR) following treatment.
- To study the association of anemia with chemotherapy related toxicity.

Review of Literature:

Anemia: definition and prevalence

Anemia is usually not a disease in itself; it is almost always a sign of an acquired or inherited abnormality. Red Blood Cells (RBC) have an average life span of 120 days and approximately 1% of RBCs are lost and replenished daily[4]. A reduction in the RBC mass resulting in anemia can occur due to increased loss, decreased production or both. Anemia can be functionally defined as an insufficient red cell mass to deliver oxygen adequately to tissues. Any of the three concentration measurements done on whole blood can be used to define anemia: the Hb concentration(g/dl), the hematocrit (Hct) or packed red cell volume(PCV) expressed in percent (%), or Red Blood Cell(RBC) concentration expressed in cells / microliter.[4] However , the most commonly used parameter is Hb level. The WHO definition also incorporates Hb level as the parameter for assessment of anemia [1]

Anemia is one of the most common hematological abnormalities encountered in clinical practice. According to western literature , significant microcytosis occurs in 3% of all patients who require hospital admission.[12] This is likely to be much higher in the Indian population , mostly due to nutritional causes[2].

Evaluation of anemia:

Measurement of the different parameters of anemia were done with manual techniques earlier. However, currently, they are measured by

electronic cell counters and Hb analyzers. From these values , Mean corpuscular volume(MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular Hemoglobin concentration(MCHC)can be measured as follows[4].

MCV : (Hct x 10)/ red cell count x 10^6; normal value : 90+/-8 femtolitre(fl)

MCH: Hb x 10/ red cell count x 10^6; normal value: 30+/- 3pg

MCHC: MCH/MCV; normal value: 33+/-2 % (2)

The electronic counters also measure an index called Red Cell distribution Width (RDW), which is a quantitative measure of the variation in red cell size [4].

Initial evaluation of anemia consists of assessing whether it is associated with other hematological abnormalities. If yes, then bone marrow examination is done to look for leukaemia, myelodysplasia, myelofibrosis, aplastic anemia. If anemia is an isolated abnormality, then the reticulocyte response is assessed . Normal reticulocyte count by light microscopy is between 0.5-1.5%.Presently, automated methods are available.

In the event of an adequate reticulocyte response, hemolytic and hemorrhagic causes are looked for. In the absence of an adequate reticulocyte response, red blood cell indices indicate the type of anemia [13] Measurement of corrected reticulocyte count(RC):[14]

Reticulocyte =% of reticulocytes in RBC population

Corrected RC=% reticulocytes x (patients' Haematocrit)/45

Reticulocyte production index= corrected RC/ maturation time in peripheral time in days*

(*Hct >/=40% :1 day, Hct 30-40%: 1.5 days, Hct 20-30%: 2 days, Hct <20%: 2.5 days)

MCV can serve as a useful index in evaluating the cause of anemia:

MCV more than 100 signifies macrocytic anemia and various causes such as folic acid and vitamin B12 deficiency have to be considered. MCV of 80- 100 signifies normocytic anemia and anemia of chronic disease is the usual diagnosis. MCV less than 80 signifies microcytic anemia and causes such as iron deficiency, thalessemia, sideroblastic anemia , lead intoxication have to be looked into.[4, 15 , 16,17]. Further evaluation consists of peripheral smear study(PS), bone marrow aspirate(BMA) to look for cell morphology , marrow response and iron stores and evaluation of the iron indices (table1, 2, 3)

Table 1: iron indices in different types of anemia [3]

Parameters	Normal range	Iron deficiency	Inflammation	Thalassaemia/ sideroblastic anemia
Serum iron	50-150microg/dl	<30	<50	Normal-high
Serum ferritin	50-200microg/ L	<15	30-200	50-300
Total iron binding capacity(TIBC)	300-360 microg/dl	>360	<300	normal
% saturation	30-50%	<10	10-20	30-80

Table 2: normal marrow response to anemia [3]

Hematocrit	Production index	Corrected reticulocytes	Marrow Myeloid:erythroid
45	1	1	3:1
35	2-3	2.5	2:1-1:1
25	3-5	4	1:1-1:2
15	3-5	4	1:1-1:2

Table 3: iron store measurements in bone marrow [3,18]

Iron stores	Marrow iron stain,0-4+	Serum ferritin,ug/L
0	0	<15
1-300mg	Trace – 1+	15-30
300-800mg	2+	30-60
800-1000mg	3+	60-150
1-2gram	4+	>150
Iron overload		500-1000

Clinical features of anemia:

1. cardiovascular and pulmonary features: patients with chronic anemia may be asymptomatic till the Hb levels drop to 8g/dl or lower[19] Common manifestations include shortness of breath, fatigue, dizziness, palpitations, and in severe cases angina pectoris, intermittent claudication and congestive heart failure[5]
2. Pallor : it is a sign of anemia and is best detected in the mucous membranes of mouth , pharynx, conjunctiva, lips and nail beds

3. skin and appendages: loss of luster and brittle hair, brittle nails, glossitis
4. neuromuscular : headache, vertigo, lack of concentration, muscular weakness
5. ophthalmologic signs: almost 20% patients have flame shaped hemorrhages, hard exudates, cotton wool spots and in some cases, papilledema is noted [20,21,22]
6. gastrointestinal symptoms such as glossitis , dysphagia

Common causes of anemia according to morphological type:[3,4]

1. Macrocytic anemia:
   - Vitamin B 12 deficiency due to various causes
   - Folic acid deficiency due to various causes
   - Combined Vitamin B12 and folic acid deficiency
   - inherited disorders of De oxy Ribonucleic Acid (DNA)synthesis
   - drug and toxin induced
   - alcoholism
   - liver disease
   - myelodysplastic syndrome
   - hypothyroidism
2. Microcytic anemia
   a. disorders of iron metabolism
   - iron deficiency anemia
   - anemia of chronic disorders

b. disorders of globin synthesis

- thalassemias
- HbE disease

c. sideroblastic anemias

d. Lead intoxication

3. Normocytic anemia

a. anemia with appropriate marrow response:

- post hemorrhagic anemia
- hemolytic anemia

b. decreased erythropoietin secretion

- anemia of renal/liver disease
- anemia of chronic disorders
- protein calorie malnutrition

c. anemia with impaired marrow response

- aplastic anemia
- bone marrow infiltrative disorders
- leukemia
- myeloma
- myelodysplastic anemias
- early iron deficiency

Variation in Hb levels with age and physiology:

It has been observed that anemia is a common occurrence in elderly persons and is probably related to decreased androgen levels or age- related decrease in stem cell proliferation [23]. However, others have suggested that increased frequency of anemia in elderly is due to the underlying disease and have reported that RBC parameters of healthy elderly individuals are within normal range[24].Similarly, at the other extreme, Hb values in infants and children differ from adults[4,25]

Blood volume adjustments also occur during pregnancy and puerperium [26].

Treatment:

Treatment of anemia is directed at the cause of anemia

Non-Hodgkins lymphoma (NHL):

Epidemiology:

Lymphomas (NHL and HL) are the fifth leading cause of cancer death and the second fastest growing cancer in terms of mortality. They constitute 4-5% of all new cancer cases [10]. The lowest incidence is reported from Asia [27].There has been a striking rise in the incidence of NHL in the past four decades and the reason is mostly unexplained [28,29]

Etiology:

Cause of NHL is mostly not known. Various genetic diseases, environmental factors and infectious agents may be involved in the causation of NHL. Inherited immunodeficiency states are associated with a 25% risk of

developing lymphoma[30] In addition, acquired immunodeficiency states such as HIV associated AIDS and organ transplantation are associated with NHL[31]

Various infectious agents are associated with NHL such as:

Epstein Barr Virus (EBV): Burkitts' lymphoma, AIDS associated lymphoma, Post transplant lymphoprolferative disorders [32,33]

Human Herpes Virus 8(HHV-8): primary effusion lymphoma, multi-centric Castlemans' disease[34].

Human T cell lymphotrophic virus (HTLV): Adult T cell leukemia / lymphoma [35,36]

Helicobacter pylori infection: Mucosa Associated lymphoid Tissue (MALT) lymphomas [37]

Campylobacter jejuni: Immuno-proliferative small intestinal disorder[38] Borrelia burgdorferi: cutaneous B cell lymphoma[39]

Chlamydia psittaci: ocular adnexal lymphoma (marzinal zone)[40] Patients with autoimmune disorders such as rheumatoid arthritis, psoriasis and Sjogrens' syndrome also have an increased risk of NHL [41] Classification of lymphomas:

The 2008 WHO classification of NHL consists of 86 distinct subtypes of lymphoma [42]. The WHO classification uses clinical, histological, phenotypic and genetic data, and provides a clinically useful system that can be applied throughout the world.

WHO classification of lymphoid neoplasms 2008:

PRECURSOR B- AND T-CELL NEOPLASMS

Precursor B-lymphoblastic leukemia/lymphoma

Precursor T-lymphoblastic leukemia/lymphoma

MATURE B-CELL NEOPLASMS

Chronic lymphocytic leukemia/small lymphocytic lymphoma

B-cell prolymphocytic leukemia

Lymphoplasmacytic lymphoma/ Waldenstroms' macroglobulinemia

Splenic marginal zone B-cell lymphoma

Hairy cell leukemia

Splenic B cell lymphoma/leukemia, unclassifiable

Splenic diffuse red pulp small B cell lymphoma

Hairy cell leukemia variant

Plasma cell neoplasms:

Monoclonal gammopathy of undetermined significance

Plasma cell myeloma

Solitary Plasmacytoma of bone

Extraosseous plasmacytoma

Monoclonal immunoglobulin deposition diseases

Extranodal marginal zone B-cell lymphoma (MALT lymphoma)

Nodal marginal zone B-cell lymphoma

Follicular lymphoma

Primary cutaneous follicle center lymphoma

Mantle cell lymphoma

Diffuse large B-cell lymphoma

T cell/ histiocyte rich large B cell lymphoma

Primary DLBCL of the central nervous system

Primary cutaneous DLBCL, leg type

EBV positive DLBCL of the elderly

DLBCL associated with chronic inflammation

Lymphomatoid granulomatosis

Primary Mediastinal (thymic) large B-cell lymphoma

Intravascular large B-cell lymphoma

ALK- positive large B cell lymphoma

Plasmablastic lymphoma

Large B cell lymphoma arising in HHV 8- associated multi-centric Castlemans' disease

Burkitts' lymphoma/leukemia

B cell lymphoma unclassifiable with features intermediate between DLBCL and Burkitts' lymphoma

B cell lymphoma, unclassifiable, with features intermediate between DLBCL and classic Hodgkins' lymphoma

MATURE T- AND NK-CELL NEOPLASMS

T-cell prolymphocytic leukemia

T-cell large granular lymphocytic leukemia

Chronic lymphoproliferative disorder of NK cells

Aggressive NK-cell leukemia

EBV positive T cell lymphoproliferative diseases of childhood

Systemic EBV + T cell lymphoproliferative disease of childhood

Hydroa vacciniforme – like lymphoma

Adult T-cell leukemia/lymphoma

Extranodal NK/T-cell lymphoma, nasal type

Enteropathy-type T-cell lymphoma

Hepatosplenic T-cell lymphoma

Subcutaneous panniculitis-like T-cell lymphoma

Mycosis fungoides

Sezary syndrome

Primary cutaneous CD30 positive T-cell lymphoproliferative disorders:

Primary cutaneous anaplastic large cell lymphoma

Lymphomatoid papulosis

Primary cutaneous peripheral T cell lymphomas, rare subtypes

Primary cutaneous gamma-delta T cell lymphoma

Primary cutaneous CD8 positive aggressive epidermotrophic cytotoxic T cell lymphoma

Primary cutaneous CD 4 positive small/ medium T cell lymphoma

Peripheral T cell lymphoma, not otherwise specified

Angioimmunoblastic T-cell lymphoma

Anaplastic large cell lymphoma, ALK positive

Anaplastic large cell lymphoma, ALK negative

IMMUNODEFICIENCY ASSOCIATED LYMPHOPROLIFERATIVE DISORDERS

Lymphoproliferative diseases associated with primary immune disorders

Lymphomas associated with Human immunodeficiency virus infection

Posttransplant lymphoproliferative disorders

Plasmacytic hyperplasia and infectious mononucleosis like PTLD

Polymorphic PTLD

Monomorphic PTLD

Classic Hodgkins' lymphoma type PTLD

Other iatrogenic immunodeficiency associated lymphoproliferative disorders

Principles of Management:

The appropriate management of patients with NHL include diagnosis, staging workup, prognostic scoring and baseline assessment of other organ systems .These include:

- Accurate diagnosis from tissue biopsy with immunohistochemical and genetic studies
- history and physical examination

- imaging and studies : chest radiograph, computed tomography(CT) scans, positron emission tomography(PET) CT scans[43]
- laboratory tests: complete blood count, biochemistry, tumor lysis parameters
- bone marrow aspirate and biopsy
- lumbar puncture in aggressive histologies and HIV associated lymphomas[10]

Staging:

The Ann Arbor staging system developed initially for staging patients with HL is used in staging NHL [44]

Ann Arbor Staging System

I.	Involvement of a single lymph node region or a single extralymphatic organ or site (IE)
II.	Involvement of two or more lymph node regions on the same side of the diaphragm (II) or localized involvement of an extralymphatic organ or site (IIE)
III.	Involvement of lymph node regions on both sides of the diaphragm (III) or localized involvement of an extralymphatic organ or site (IIIE) or spleen (IIIS) or both (IIISE)
IV.	Diffuse or disseminated involvement of one or more extralymphatic organs with or without associated lymph node involvement. Bone marrow and liver involvement are always stage IV

Identification of the presence or absence of symptoms should be noted with each stage designation: A, asymptomatic; B, fever, sweats, weight loss greater than 10% of body weight.

Prognostic index:

The international prognostic index (IPI) is a valuable tool to stratify patients with NHL into different risk groups [45,46]This includes five factors which have an equal and independent effect on survival. These factors are:

Age > 60 years

Serum lactate dehydrogenase (LDH) >upper limit of normal

Performance status (PS) > 2

Advanced stage disease

> 2 extra nodal sites

The IPI is useful in risk stratifying almost all subtypes of lymphoma. However, it is less useful in certain types of lymphoma such as follicular lymphoma (FL) and mantle cell lymphoma (MCL) where separate predictive models are available such as Follicular Lymphoma International Prognostic Index (FLIPI) and Mantle cell lymphoma International Prognostic Index (MIPI) respectively.

The FLIPI consists of the following factors: age >60years, stage 3 and 4, Hb level<12g/dl, > 4 nodal areas involved and serum LDH above upper limit of normal [11] The MIPI consists of : age, PS , Serum LDH and Total White Blood Cell (WBC) count. [47]

Treatment:

The treatment recommendation for NHL is made after considering patient related factors (PS), type of lymphoma and disease stage. Most subtypes of NHL are treated with CHOP or CHOP-like chemotherapy regimens with or without rituximab(in B cell lymphomas) /Radiotherapy [48,49,50,51,52]

Anemia in cancer:

Anemia is a common finding in cancer patients [6]. Anemia occurs in over 30% of cancer patients at any point in time, and its incidence increases with treatment and as disease progresses. Causes of anemia in cancer patients are:

1. Related to the patient :hemoglobinopathies, gastrointestinal problems
2. Secondary to the disease: bone marrow infiltration, bowel resection, hypersplenism, poor nutrition
3. Related to therapy : secondary to radiotherapy or chemotherapy

A major symptom of organ disturbance is fatigue. In oncology, this symptom ranks first among patient complaints and correlates with the hemoglobin level.Almost one third of patients become anemic after three cycles of chemotherapy .Several studies have shown that improving the hemoglobin level can improve quality of life in cancer patients and this is most marked when hemoglobin levels are increased to 11- 12 g/dL[53]

Anemia in lymphoid malignancies:

Anemia is reported in 40-80% patients with lymphoid malignancies [56] Several studies have looked into the prognostic significance of bone marrow involvement in NHL. However, very few studies have investigated the impact of anemia at diagnosis of NHL [57,58,59]

The impact of anemia as a prognostic factor in Hodgkin lymphoma is well known. Anemia can be an initial feature in approximately 40% of patients with HL. It is usually seen in advanced stages and in association with B symptoms .The anemia is usually a mild normochromic, normocytic in type with hemoglobin (Hb) levels between 10 and 12 g/dl. The inflammatory response around the Reed-Sternberg cell is part of HL .As a result of the reactive cells of the microenvironment, high levels of cytokines, such as IL-6, IL-10, and the chemokine thymus and activation-regulated cytokine (TARC) among others are secreted. These cytokines result in the development of systemic symptoms and laboratory abnormalities that are correlated with disease prognosis. Hence, anemia is incorporated in the prognostic scoring in HL. IL-6 is among the cytokines most strongly associated with anemia.[60,61,62]

Anemia in NHL: Prevalence and prognostic significance

Anemia is reported in approximately 50 % of NHL patients at presentation. [7,57,58,59,63] In one study, the prevalence of anemia in NHL was 44.4 percent. Women had a higher prevalence of anemia than men, but the severity was higher in men at the beginning and at the end of treatment.The

most common anemia was anemia of chronic disease (53.8%), followed by anemia due to combined factors (anemia of chronic disease + iron deficiency anemia or anemia of chronic disease + hemolytic anemia). This was followed by anemia due to iron deficiency and hemolytic anemia. The authors concluded that prevalence of anemia as a consequence of the disease is high in lymphoproliferative disease and that chemotherapy had a minimal impact on anemia. In fact, there was a decline in the prevalence of anemia in NHL patients (44% Vs 21%) at the end of chemotherapy. However, the severity of anemia increased after chemotherapy in patients who had anemia at baseline [63]

A study of 1077 NHL patients found that anemia was the most common hematological finding at diagnosis and was present in 32% of patients and it varied according to the subtype of lymphoma. The study also found that anemia was more common in patients with bone marrow involvement. Anemia was also significantly associated with certain patient characteristics. They found that a significantly larger percentage of anemic patients had the following characteristics: ≥60 years of age (39%), stage III or IV disease (37%), elevated LDH (47%), presence of B symptoms (61%), and ≥2 extra nodal sites (47%). They also found that the rate of complete response (CR) was lower in patients with anemia and in an univariate analysis, the overall survival (OS) and progression-free survival (PFS) were significantly shorter for anemic patients. [7] It was significantly associated with decreased PFS in

Small lymphocytic lymphoma (SLL), Mantle cell lymphoma (MCL) ,Diffuse large B cell lymphoma(DLBCL) and other high grade NHL and with shorter OS in all subgroups except marginal zone lymphoma. [7] A study of anemia in Intermediate Grade NHL found anemia to be associated directly with other prognostic factors such as age>60, extra nodal sites>/=2, stage 3 or 4, elevated LDH and B symptoms. It was a retrospective study on the baseline prevalence and prognostic value of anemia in a group of patients with IGNHL. Thirty-five percent of patients in the study were anemic at baseline. They found that the prevalence of anemia varied with histological subtypes of NHL. In this study of intermediate-grade NHL, a larger percentage of anemic patients had the following histology subtypes: large cell immunoblastic(56%) and large cleaved or noncleaved cell/diffuse (38.9%)[64].

There is a conflict in published literature regarding the relationship between anemia and histology subtypes. This may result from the fact that these studies had a different patient distribution for different NHL grades. Moullet et al reported that the incidence of anemia was low in indolent lymphomas (17% in follicular and 19% in marginal zone lymphomas) with the exception of small lymphocytic or lymphoplasmacytoid (37%)[7] Conlan et al reported that patients with high-grade lymphoma have a higher prevalence of anemia than patients with intermediate-grade and low-grade lymphomas.[65] On the other hand, Bloomfield et al found no significant difference among histology types[66]. Few studies have indicated that bone marrow involvement

was significantly associated with anemia [7,66]Others have reported that even though bone marrow involvement is associated with anemia, it may not be at a significant level [65,67]. Many studies have determined that anemia prior to chemotherapy or radiotherapy can have a negative impact on survival and decreased response to therapy. Morel et al evaluated the factors associated with complete remission in patients with NHL and acute lymphoblastic leukemia and concluded that age >40, high LDH level, low Hb level (<10 g/dl), and two or more extra nodal sites had a poor response to treatment [59].

Morrow et al also concluded that there is a significant association between baseline anemia and response to chemotherapy. Anemia was found to be associated with female sex , elevated LDH , presence of B symptoms, advanced stage NHL (stage III or IV) and large cell diffuse histology. [64]

These results indicate that the presence of baseline anemia was a significant risk factor for no response to CHOP chemotherapy even after controlling for a subset of the five commonly used factors in the International Prognostic Index. [7,65,66 ,67,68,69, 70]

Another study evaluated anemia in 59 patients with diffuse large B cell lymphoma. It was a retrospective study to determine the response to RCHOP therapy in DLBCL patients conducted between 2004 and 2009. PFS was measured from last RCHOP dose to the date of progression. Overall survival was measured from diagnosis to death or until December 2009. The median PFS for 54 patients who reached remission was 28 months (0.7-57+).and

median OS of all patients was 30 months (0.8-60+). They found that 46% patients were anemic during treatment and had a lower rate of CR (59% vs. 81%). Anemia was also associated with a higher mortality rate (37% vs. 16%), and median PFS [18 (0.7-52+) vs. 30 (2-57+) months] and OS [17 (0.8-59+) vs. 34 (5-60+) months] were shorter when compared to non-anemic patients.[71]

A study from Delhi, India prospectively evaluated the prevalence of anemia in patients with lymphoid malignancy[72]. They evaluated newly diagnosed patients of lymphoid malignancy [non-Hodgkin lymphoma (NHL), Hodgkin lymphoma (HL), and chronic lymphocytic leukemia (CLL)] for anemia. The prevalence of anemia in patients with lymphoid malignancy was found to be 42.41% .It was multi factorial in 39.13 % patients. Anemia of chronic disease was the commonest cause of anemia and was present in 71.74% patients. This was followed by nutritional anemia (iron, vitamin B12 and folic acid deficiency) in 47.83% and Autoimmune hemolytic anemia in 10.87% of patients. The authors concluded that although anemia of chronic disease is the commonest cause of anemia in patients with lymphoid malignancy, it is multi factorial in a large number of patients and hence it is important to rule out other causes of anemia.

Correction of anemia needs correction of the cause. Nutritional anemia needs correction of the nutritional deficiency (iron, vitamin B12, Folic acid). Blood transfusion may be needed in severe anemia (Hb< 7g/dl). Anemia of

chronic disease may respond to erythropoietin though there are concerns regarding the negative impact on survival reported with erythropoietin analogues. [6,8,71].

Anemia is a common hematological abnormality in NHL. The cause of anemia in NHL is usually multi factorial. The exact relevance of anemia in the setting of NHL is not known. However, it is likely that anemia may have a significant impact on the prognosis of NHL. Very few studies, and hardly any in India, have looked into the frequency and prognostic significance of anemia in NHL. Such studies are particularly important in the Indian setting where the prevalence of nutritional anemia is much higher and the impact of anemia, if any, on the overall prognosis may be much more relevant. Anemia, especially nutritional anemia is easily correctable, and hence, it is of utmost importance to know the impact of anemia on the prognosis or treatment related toxicity in NHL. The present study may help to throw some light on this aspect and have relevance on the management of patients with NHL, particularly in the Indian context.

Materials and Methods:

This study is a prospective single - center study.

Period of study: June 2011 to November 2012

All patients with newly diagnosed NHL at our institute were screened for anemia. Anemia was defined as a hemoglobin level less than 13g/dl in men and 12 g/dl in women as per WHO definition of anemia.

Inclusion criteria:

- Age more than or equal to 15 years
- Newly diagnosed patients with NHL (not previously treated)

Exclusion criteria:

- Age less than 15 years
- Patients with chronic kidney disease
- Patients with known hemoglobinopathies
- Patients who have received Blood transfusions or have taken iron, folic acid or vitamin B12 supplements in the previous two weeks
- Patients with relapsed NHL
- Patients who have received treatment for NHL
- Patients with lymphoblastic lymphoma were treated as per Acute lymphoblastic leukemia protocol and were excluded from the study

Patients included in the study were subjected to the following investigations to determine the cause of anemia:

Peripheral blood studies:

- Complete Blood Count(CBC) including total white blood cell count , hemoglobin , platelet count and differential count.
- RBC indices: MCV, MCH , MCHC and hematocrit
- Renal function test and Liver function test
- Reticulocyte count, corrected reticulocyte count and reticulocyte index
- Peripheral blood smear study(PS)
- Direct Coombs' test
- Iron indices which includes
- Serum iron (SI)
- Serum ferritin
- Serum Total iron binding capacity (TIBC)
- Transferrin saturation
- Patients found to have macrocytic anemia on PS with a reticulocyte count suggestive of hypoproliferative anemia also underwent testing for serum vitamin B12 and folic acid levels
- Bone marrow aspiration and biopsy studies:
- morphology
- Bone marrow iron study

Other studies:

- C reactive protein (CRP)
- Routine Stool examination
- occult blood study
- gastrointestinal endoscopy as indicated in patients with iron deficiency anemia

All patients were staged as per Ann Arbor staging and IPI score was determined for each patient. Patients were subjected to complete re-staging investigations after four cycles of planned chemotherapy to assess response.

Complete response (CR) , Partial response (PR) and stable disease(SD) were defined as per the International Working Group (IWG) on Lymphoma Response Evaluation Criteria.[73]In patients subjected to PET CT scans as part of their staging evaluation, Cheson Criteria for Lymphoma was applied .[74]

Patients were assessed for toxicity after each cycle of chemotherapy and treatment related toxicity was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI- CTC AE) [75]

Definitions:

Reticulocyte index (RI) was calculated in all cases. If RI was less than 2.5, the patient was diagnosed to have a hypoproliferative anemia (normocytic / normochromic) or maturation disorder (micro/ macrocytic)

depending on the PS study. If RI was more than or equal to 2.5, the patient was evaluated for hemorrhage or hemolysis.[3]

- Iron deficiency anemia(IDA) was diagnosed by the following parameters:
- transferrin saturation <20%
- Serum ferritin (SF) <15 microgram/L and
- Total iron binding capacity (TIBC) >360ug/dl
- Vitamin B12 deficiency has been defined as a serum level < 100ngm/ml in the presence of macrocytosis
- Folic acid deficiency has been defined as a serum level <4mcg/ml in the presence of macrocytosis
- Autoimmune hemolytic anemia (AIHA) was diagnosed if Coombs' test was positive and hemolysis was evident on the peripheral smear
- Anemia of chronic disease(ACD) was diagnosed by
- transferrin saturation of 10-20% ,
- SF between 30-200ug/L and
- TIBC of <300 microgram/dl
- IDA+ACD was diagnosed if
- transferrin saturation was <20
- TIBC was <300microgram/dl and
- SF was <30 microgram /L.

Red cell indices and marrow iron stores were evaluated as per standard reference ranges[3]

The following analyses were planned for the study:

Frequency of anemia in patients with newly diagnosed NHL at our Institute will be analyzed with age and sex distribution.

- Frequency of anemia in different subtypes of NHL
- Distribution of different patterns of anemia in patients with NHL
- causes of anemia in patients with NHL
- frequency of anemia in different stages of NHL
- Any significant association of anemia with bone marrow involvement, B symptoms or elevated LDH in NHL (chi-square test)
- Association of anemia with attainment of complete response and partial response evaluated with chi-square test
- Association of anemia with chemotherapy related toxicity (chi-square test/ Fishers' exact test as applicable.)

(*p value < 0.05 was considered to be statistically significant*)

Results and observations:

A total of two hundred (200) patients with newly diagnosed NHL were screened for anemia. Anemia was present in 144 / 200 (72%) patients.

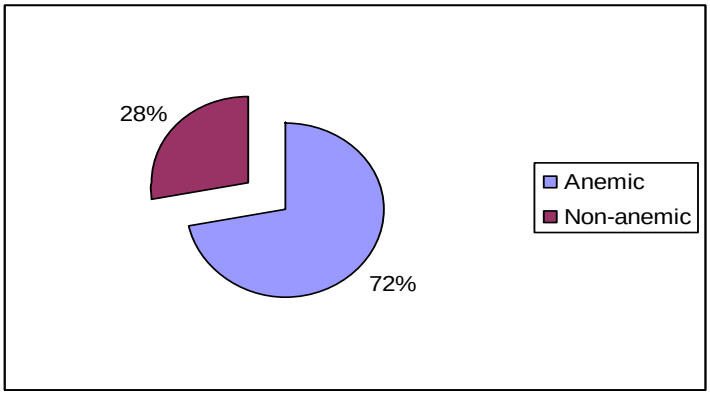


Figure 1 Frequency of Anemia

Age-wise distribution of anemia in NHL patients (n=144):

15-40 years: 28 /144(19.4%)

41-60 years: 40/ 144(27.7%)

> 60 years: 76/144(52.7%)

Mean age of the study population was 54.2 years. Median age was 59 years (range 18-75). Almost 53% patients with anemia were > 60 years of age. **There was a statistically significant association of anemia with age > 60 years (*p value 0.02, chi square = 7.83*) in patients with NHL.**

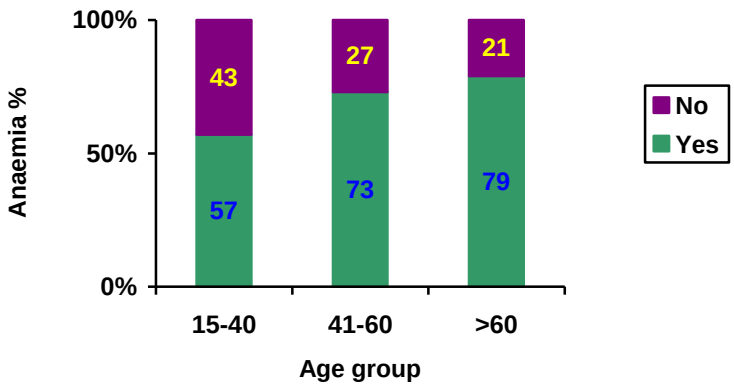
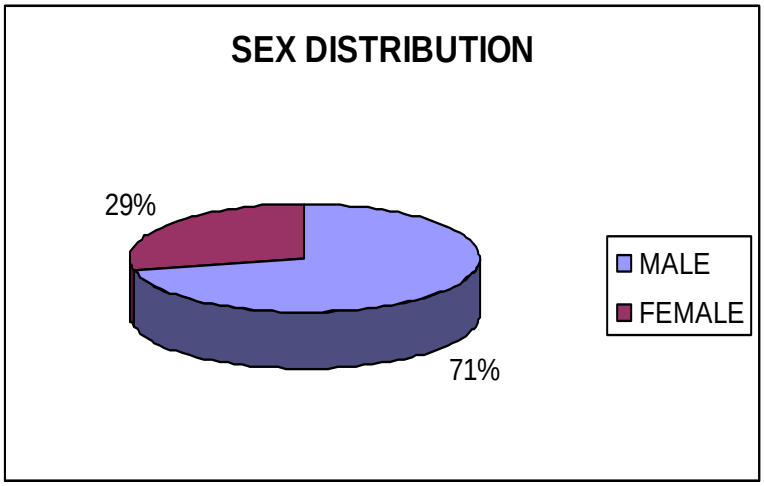


Figure 2: distribution of anemic patients in different age groups (n= 200)

Sex distribution:

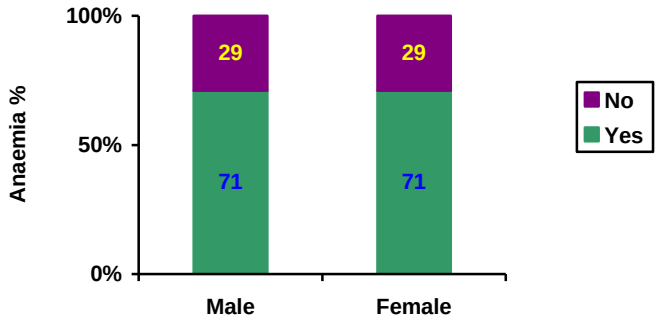
Among the 200 patients included in the study, 142 (71%) were males and 58 (29%) were females.



Among 144 patients diagnosed with anemia, 102 (71%) were males and 42 were females (29%). Male: female ratio was 2.4:1 in anemic patients.

There was no significant association of anemia with female sex (***p=0.93***, chi square=0.01, table 1 and bar diagram below).

	Male	Female	%of males	% of females
Anemic n=144	102/144	42/144	71%	29%
non anemic n=56	40/56	16/56	71.4%	28.6%



Subtypes of NHL:

Distribution of different subtypes of NHL among the 200 patients:

Diffuse large B cell lymphoma (DLBCL): 129/200(64.5%)

Follicular lymphoma (FL): 35/200(17.5%)

Composite lymphoma (DLBCL+FL): 6/200 (3%)

Mantle cell lymphoma (MCL): 10/200(5%)

Others: 20/200(10%), which includes the following:

Small lymphocytic lymphoma (SLL): 3/200 (1.5%),

Angioimmunoblastic lymphoproliferative disease (AILD): 1 (0.5%)

Anaplastic large cell lymphoma (ALCL): 6/200 (3%),

Extranodal NK T cell lymphoma: 2/200 (1%),

Splenic marzinal zone lymphoma(SMZ): 1/200 (0.5%),

Lymphoplasmacytic lymphoma: 2/200 (1%),

Cutaneous T cell Lymphoma(CTCL): 2/200 (1%),

Burkitts' lymphoma (BL): 3/200 (1.5%)

Distribution of different subtypes of NHL in anemic patients (n=144):

DLBCL: 90 (62.5%)

FL: 29(20%)

Composite lymphoma (DLBCL+FL): 6 (4%)

MCL: 8(5%)

Others: 11/144(7%), which includes the following:

SLL: 2 (1.3%),

AILD: 1 (0.6%),

ALCL: 2 (1.3%)

,Extra nodal NK T cell lymphoma: 1 (0.6%),

SMZ: 1 (0.6%),

Lymphoplasmacytic lymphoma: 2 (1.3%)

CTCL: 1 (0.6%)

BL: 1 (0.6%)

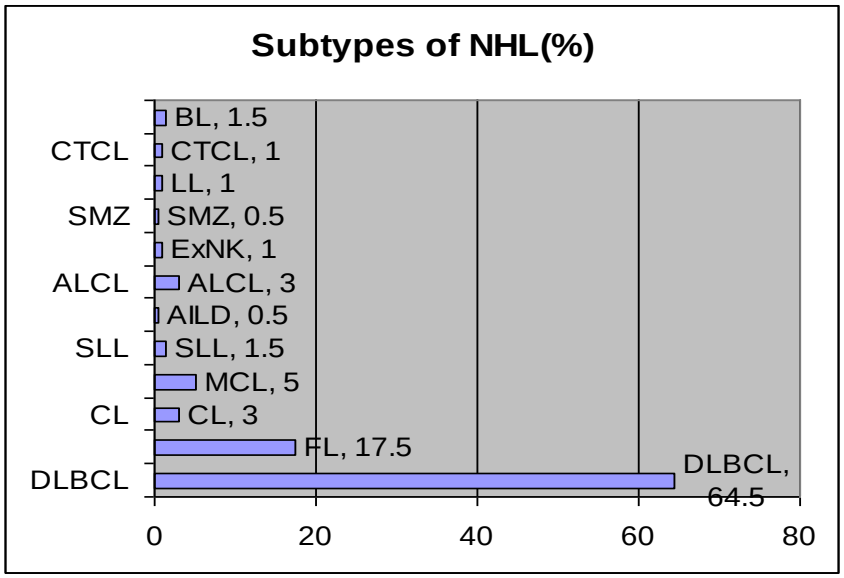


Figure 3 : distribution of different subtypes of NHL in the study population

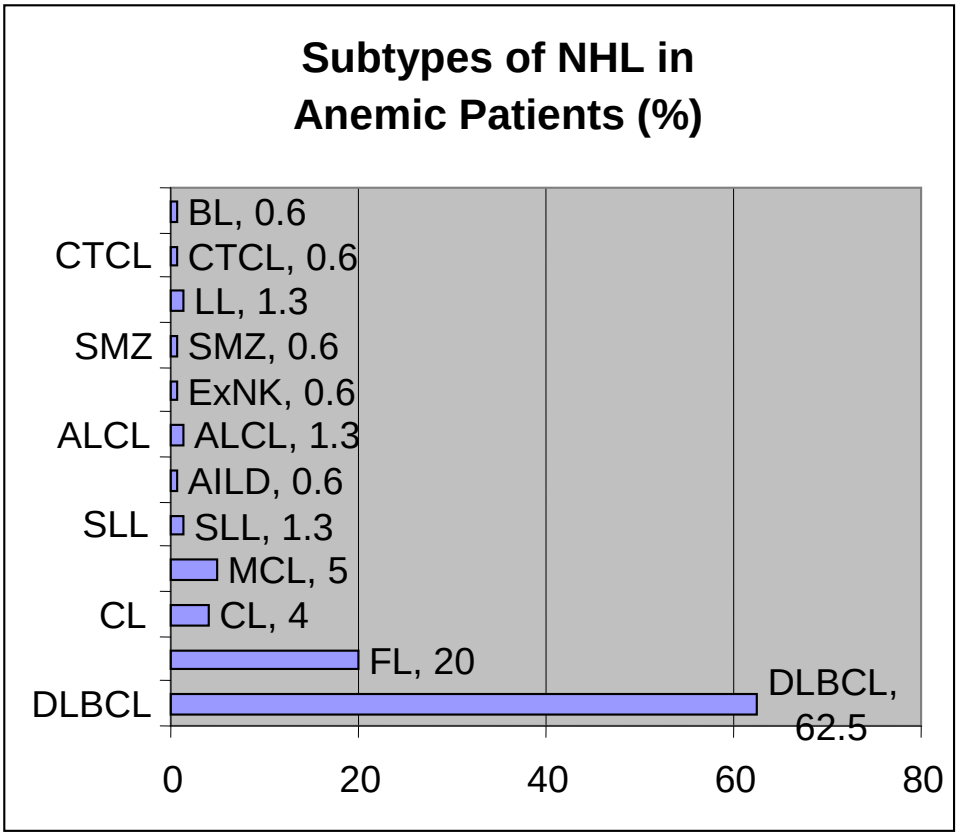


Figure 4:distribution of different subtypes of NHL in anemic patients

Anemia was commonly seen in high grade lymphomas. DLBCL was the type of lymphoma seen in 62.5% of anemic patients followed by follicular lymphoma, which was observed in 20% of patients. It was also observed that 8/10 patients with MCL and 2/3 patients with SLL had anemia. However, ***the association of DLBCL and FL with anemia was not found to be statistically significant, (p=0.34, chi square= 0.90 and p=0.11, chi square=2.48 respectively, tables 2a and 2b)***

Table 2a*: DLBCL histology was not significantly associated with anemia. (p= 0.34)*

	Anemia present	**Anemia absent**	**Total patients**
DLBCL	90	39	129
Not DLBCL	54	17	71
Total patients	144	56	200

Table 2b:*FL histology was not significantly associated with anemia (p=0.11).*

	Anemia present	**Anemia absent**	**Total no of patients**
FL	29	6	35
Not FL	115	50	165
Total patients	144	56	200

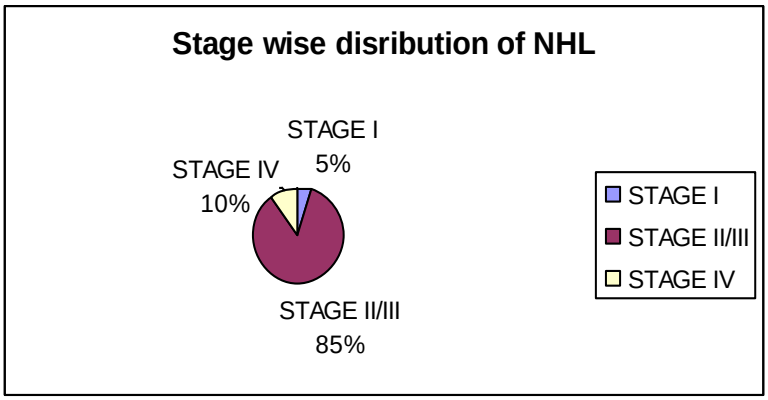
No particular subtype of NHL was found to be significantly associated with anemia in the study.

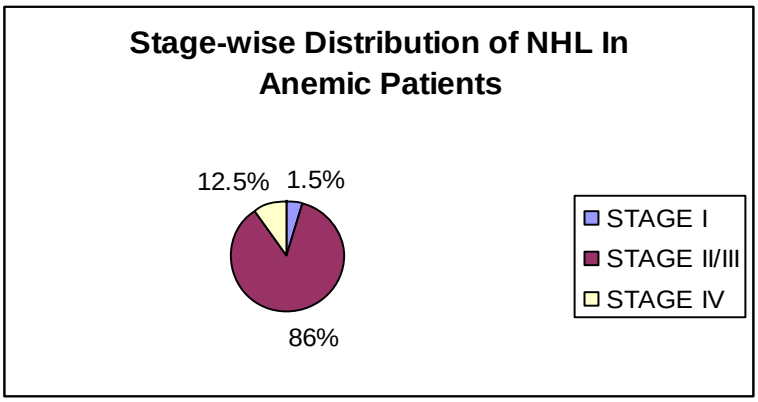
The stage-wise distribution of all patients with NHL was as follows:

Stage	No of patients (n=200)
I	10 (5%)
II/ III	170 (85%)
IV	20 (10%)

Stage wise distribution in anemic patients:

Stage	No of patients(n=144)
I	2(1.5%)
II/III	124(86%)
IV	18(12.5%)





Thus, majority of the patients (86%) were in stage II and stage III.

Bone marrow involvement was seen in 13 patients (among 20 patients with stage IV disease). All of these 13 patients had anemia. The association of bone marrow involvement with anemia was found to be statistically significant (*p=0.02, Fisher exact test*)

Table 3: association of BM involvement and anemia (p=0.02)

Bone marrow	**Anemia present**	**Anemia absent**	**Total patients**
Involved	13	0	13
Not involved	131	56	187
Total patients	144	56	200

B symptoms were present in 25 %(50/200) patients. Of these, 38 patients (76%) were anemic and 12(24%) patients were non anemic. ***However, the association of anemia with B symptoms was not statistically significant*** .

(p=0.47, chi square=0.53, table 4)

B symptoms	**Anemia present**	**Anemia absent**	**Total patients**
Present	38	12	50
Absent	106	44	150
Total patients	144	56	200

Elevated LDH was found in 90% of patients with anemia (130/144). **This association was not statistically significant.**

(***p=0.83,chi square=0.04 table5)***

Elevated LDH	Anemia present	Anemia absent	Total patients
Present	130	50	180
Absent	14	6	20
Total patients	144	56	200

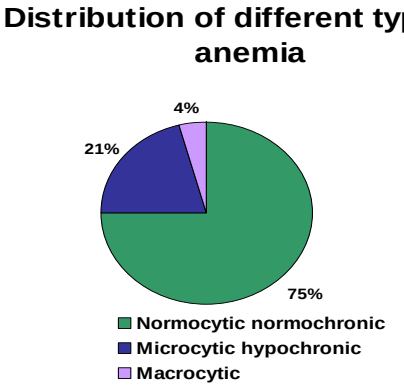
The mean hemoglobin (Hb) was 10.7g/dl and median hemoglobin was 10.4 g/dl (range 6.5-14.5).

The distribution of different types of anemia was as follows:

Normocytic normochromic anemia was seen in 108/144 patients (75%)

Microcytic hypochromic anemia was seen in 30/144(21%) patients.

Macrocytic anemia was seen in 6/144 patients (4%)



Thus**,** ***normocytic normochromic anemia was the most common morphologic type of anemia observed in the study***

Etiology of anemia:

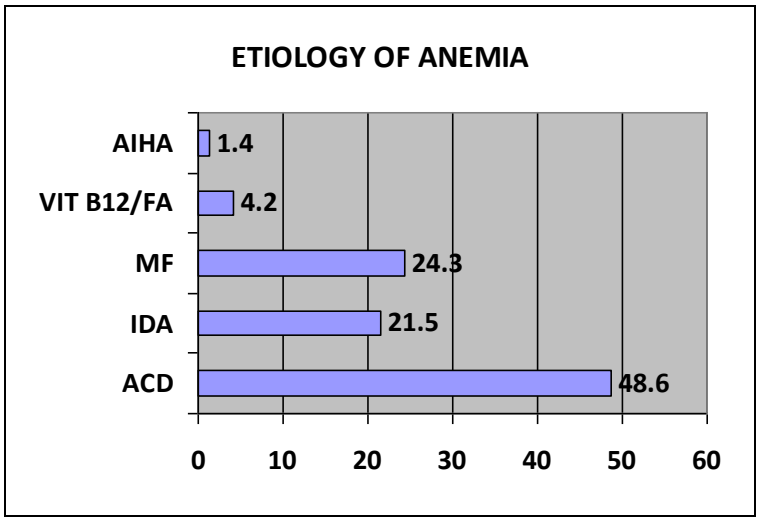
Anemia of chronic disease (ACD): 70(48.6%)

Iron deficiency anemia (IDA): 31(21.5%)

Multi-factorial (IDA+ ACD): 35(24.3%)

Vitamin B12 and folic acid deficiency: 6(4.2%)

Autoimmune haemolytic anemia(AIHA) : 2(1.4%)



Anemia of chronic disease was the most common cause of anemia. This was followed by multi factorial causes and nutritional causes**.** ***CRP was elevated in all cases diagnosed to have ACD.***

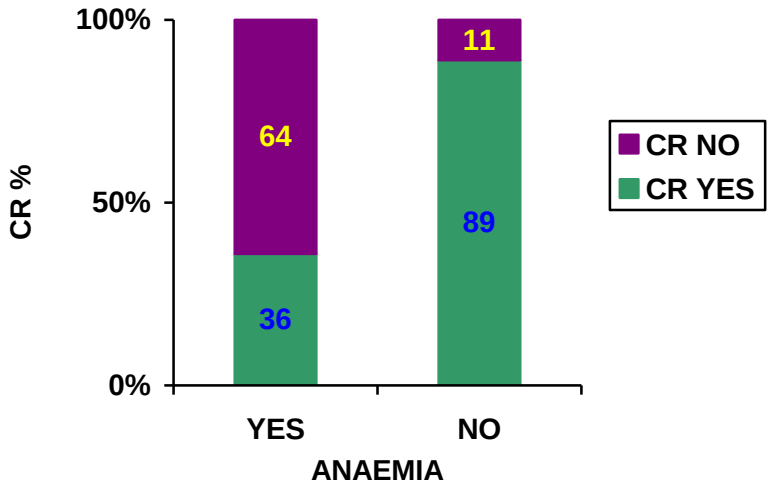
Anemia and response to chemotherapy:

Patients were reassessed after 4 cycles of chemotherapy for response with standard imaging, laboratory investigations and bone marrow study (if involved initially). Of all patients included in the study (n=200), 102 patients (51%) attained CR. Of these 102 patients, 52 patients (32%) were anemic and 50 patients were not anemic (89%). Thus, ***CR in patients with anemia was***

much less compared to those without anemia and this association was statistically significant on univariate analysis

(p<0.001, chi square=45.6, table 6)

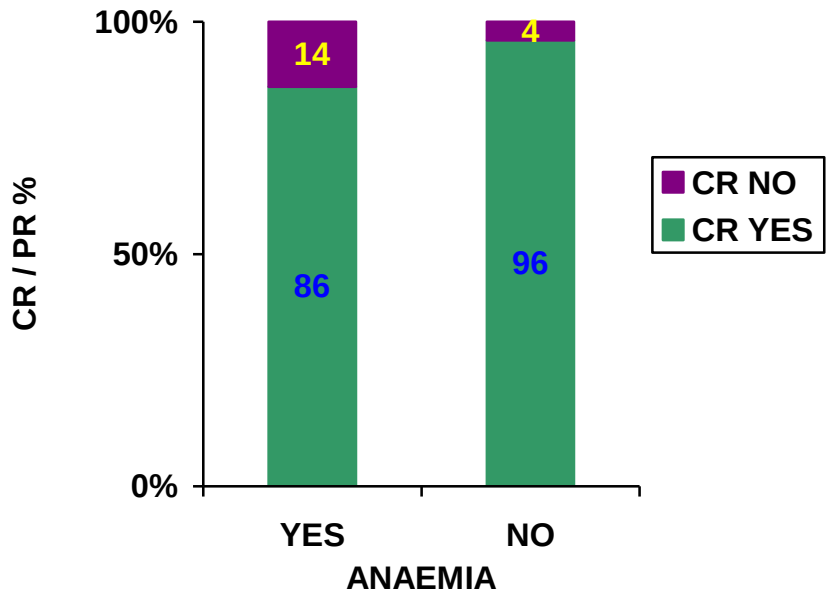
After 4 cycles	Anemia present	Anemia absent	Total patients
CR present	52	50	102
CR absent	92	6	98
Total patients	144	56	200



When combined CR and PR responses were analyzed, it was observed that ***there was a statistically significant association between anemia and response to chemotherapy***

(p=0.04, chi square=4.38, table 7)

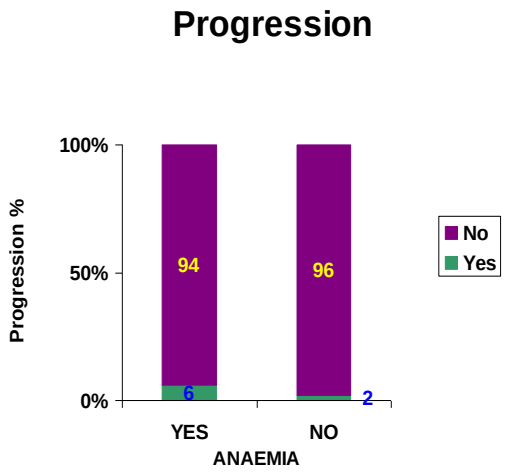
	Anemia present	Anemia absent	Total patients
CR/PR present	124	54	178
CR/PR absent	20	2	22
Total patients	144	56	200



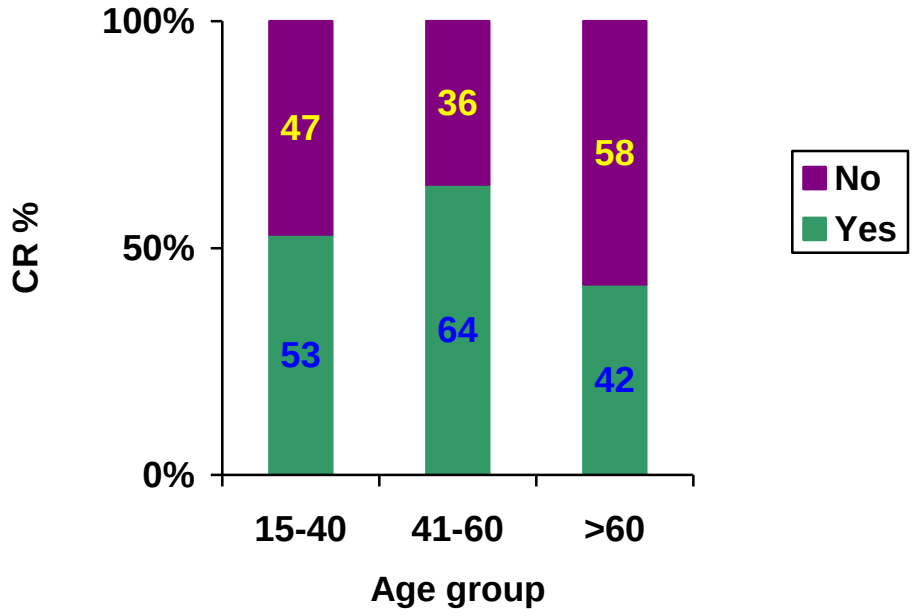
Among 200 patients, twelve patients had stable disease and all had anemia at diagnosis. Ten patients had progressive disease of which eight patients had anemia. However, ***the association of progressive disease with anemia was not found to be statistically significant***

(p=0.82,chi square= 0.05,table 8)

	Anemia present	**Anemia absent**	**Total patients**
Progression	8	2	10
No progression	136	54	190
Total patients	144	56	200



In an univariate analysis, we a found **significant association of older age with attainment of CR.** ***Persons older than 60 years had lesser incidence of CR than those below 60 years (p=0.03,chi square=7.05).***



Age> 60 years and presence of anemia were both significantly associated with lesser frequency of CR. Hence, multi factorial logistic regression analysis was done to determine the significance of each factor in association with attainment of CR. It was found that both age > 60 years and presence of anemia were independently associated with a higher risk of not attaining CR. Risk of not attaining CR was 17 fold higher in patients with anemia and 9 fold higher in patients aged more than 60 years compared to younger age groups. Hence, anemia was significantly associated with a higher risk of lesser response to chemotherapy and the association was independent of age.

Multi factorial logistic regression analysis:

factor	**number**	**Odds ratio of not attaining CR**	**95% confidence interval**
Age group(years)			***p<0.001***
15-40	28	1.00	
41-60	40	1.38	0.5-3.6
>60	76	**9.19**	***3.5-23.7****
Anemia absent	56	1.00	
Anemia present	144	**17.19**	***6.3-46.4****

* significant association with not attaining CR

Anemia and treatment related toxicity:

Treatment related toxicity was seen in all patients. (n=200)

The most common toxicity was neutropenia (198/200 patients) if all grades were considered. The two patients who did not have neutropenia were patients with CTCL who were treated with total skin electron beam therapy(TSEBT). The common toxicities observed were:

Neutropenia: Grade 3 and above: 80/200(40%)

Thrombocytopenia: 50/200(25%), all were grade 1/2

Febrile neutropenia : 70/ 200(35%)

Chemotherapy induced nausea and vomiting (CINV): 65/200(32.5%)

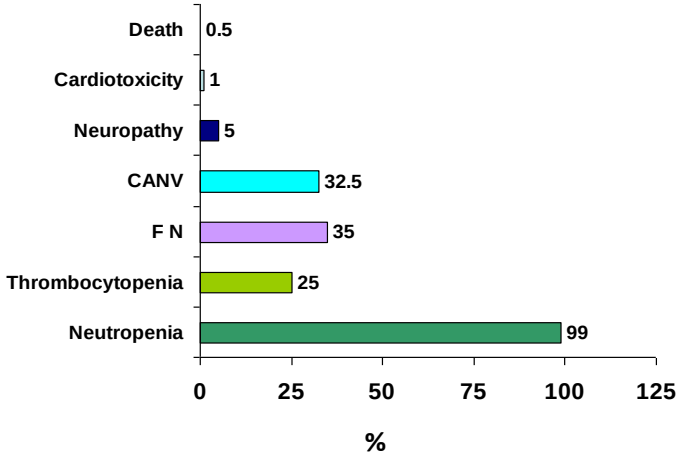
Neuropathy (vincristine related): 10 /200patients (5%)

Cardio toxicity (anthracycline related, defined by a drop in ejection fraction by > 10% from baseline or clinical features of heart failure): 2/200(1%)

Nephrotoxicity: none

Hepatotoxicity: none

Toxicities



Grade 3 or higher toxicity was seen in 80/200 patients (40%) and these were neutropenia / febrile neutropenia .Among cases with CINV (n=65), 50 patients had grade 1 and 15 patients had grade 2 toxicity. All cases of neuropathy were grade 1.Vincristine dose was reduced/ omitted after documentation of neuropathy. Among two patients who had cardiotoxicity, one patient had grade 1 and the other had grade 2 toxicity.Two deaths occurred during the study period, the cause of death being progressive Burkitts' lymphoma in one patient and neutropenic sepsis in the other. Hence, the treatment related mortality was 1/200 patients (0.5%).

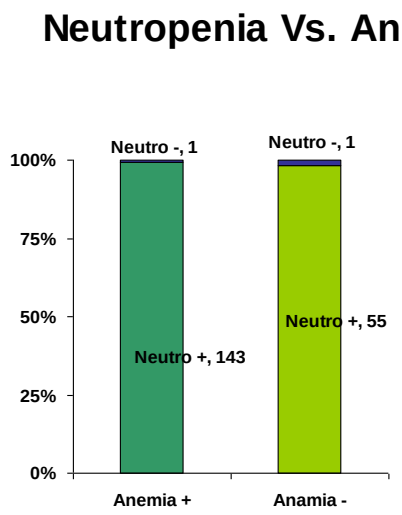
Of patients who had neutropenia (n=198), 143 patients were anemic and 55 patients were not anemic. ***There was no significant association of***

anemia with neutropenia or grade 3 and higher toxicity including febrile neutropenia.

All anemic patients with Hb <7 g/dl were transfused with packed red cells prior to initiation of chemotherapy as per our institute protocol. *There was no worsening of anemia by >1g/dl in patients with baseline anemia after chemotherapy.* Patients who did not have anemia at diagnosis did not develop anemia after chemotherapy.

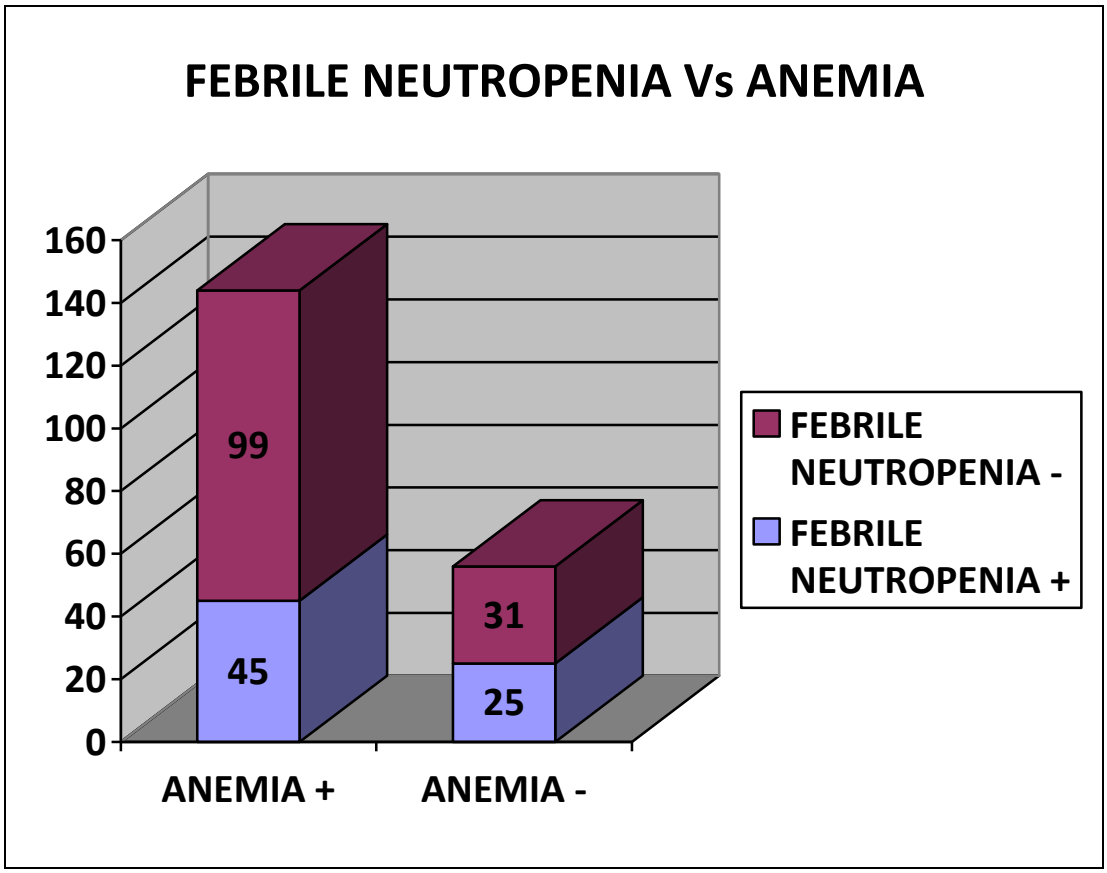
(p=0.48 chi square=0.49 ,table 9: neutropenia and anemia)

	Anemia present	**Anemia absent**	**Total patients**
Neutropenia	143	55	198
No neutropenia	1	1	2
Total patients	144	56	200



(p=.07, chi square=3.18, table 10: febrile neutropenia and anemia).

	Anemia present	**Anemia absent**	**Total patients**
Febrile neutropenia	45	25	70
No febrile neutropenia	99	31	130
Total patients	144	56	200



Summary of results:

Total number of patients analyzed: 200

Patients with anemia: 144(72%); mean Hb: 10.7/dl; Median Hb:10.4 g/dl

Most common pattern of anemia: normocytic normochromic anemia

Most common cause of anemia: anemia of chronic disease

Median age was 59years;Age >60 years significantly associated with anemia

Male: female ratio in anemic patients is 2.4:1. No significant association with female sex.

No significant association of anemia with specific subtype of NHL, B symptom, elevated LDH or chemotherapy related toxicity.

Significant association of anemia was found with bone marrow involvement.

Significant association of anemia with attainment of response to treatment.

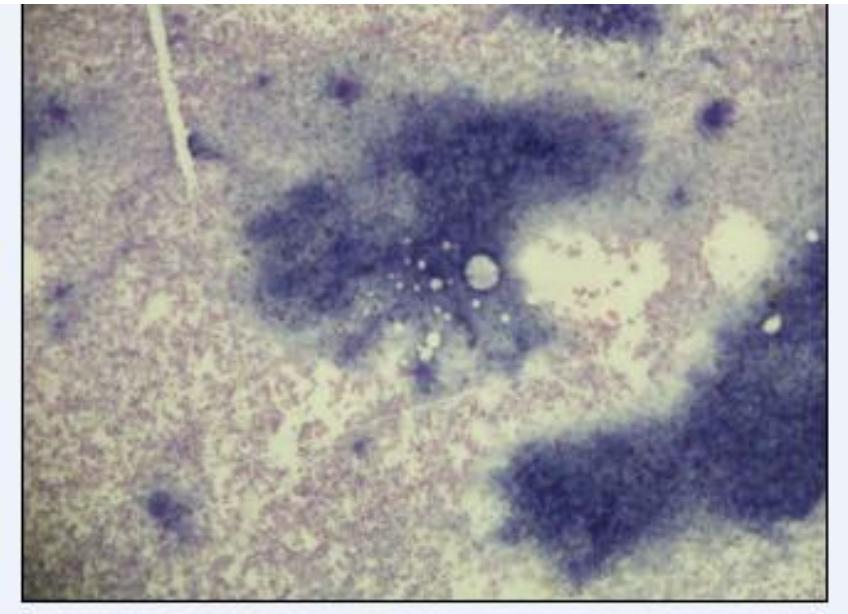


Figure A:Hypercellular bone marrow as a response to anemia

(patient: serial no:103)

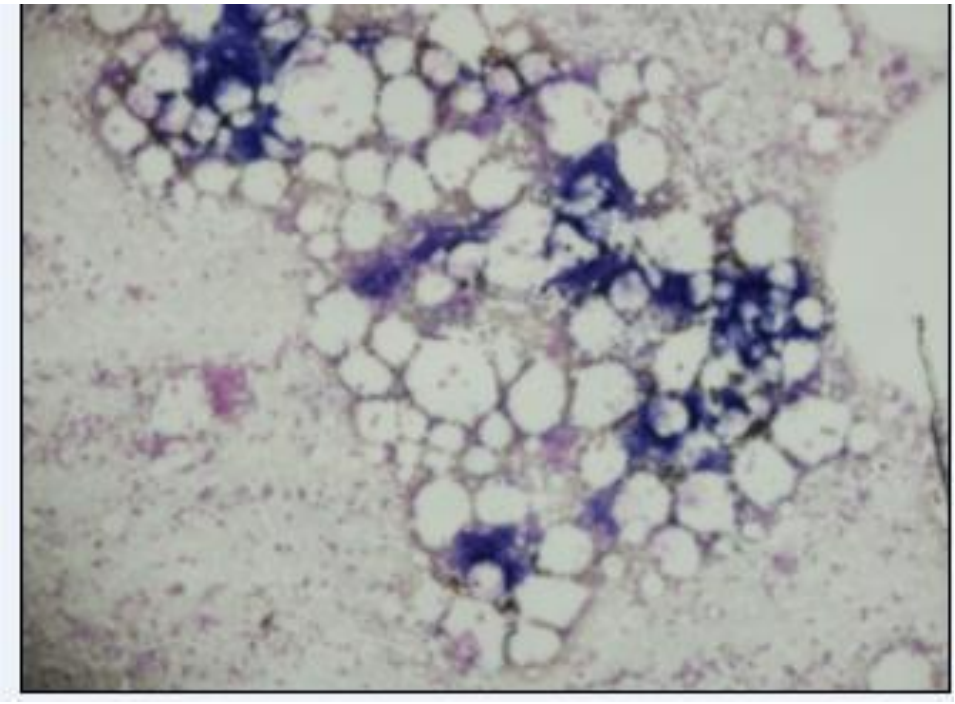


Figure B: bone marrow in a patient with hypoproliferative anemia

(patient :serial no 56)

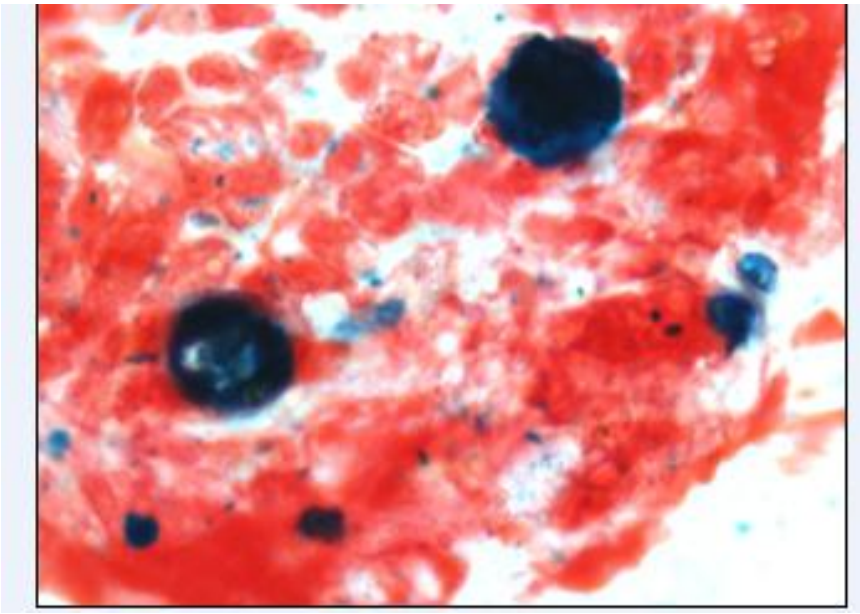


Figure C: bone marrow iron study(2+)(patient :serial no 50)

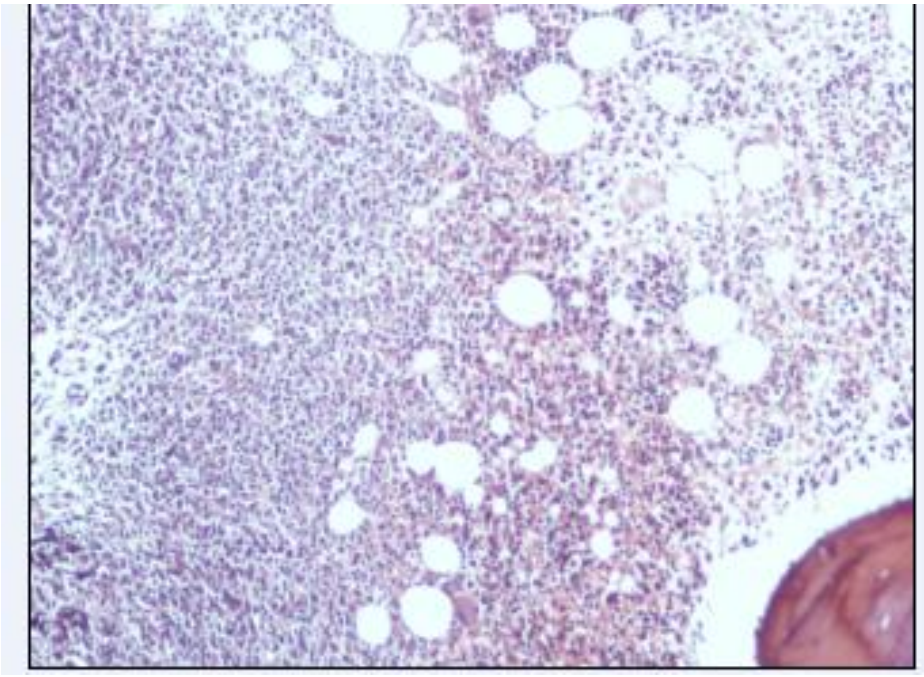


Figure D:NHL with bone marrow infiltration (patient:serial no 8)

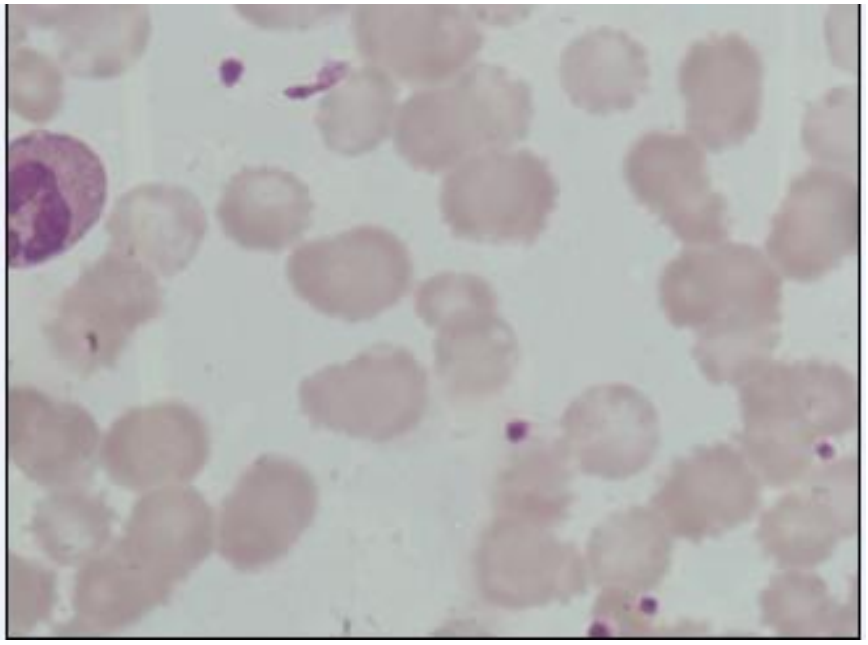


Figure E: PS of a patient showing macrocytic anemia

(patient:serial no 102)

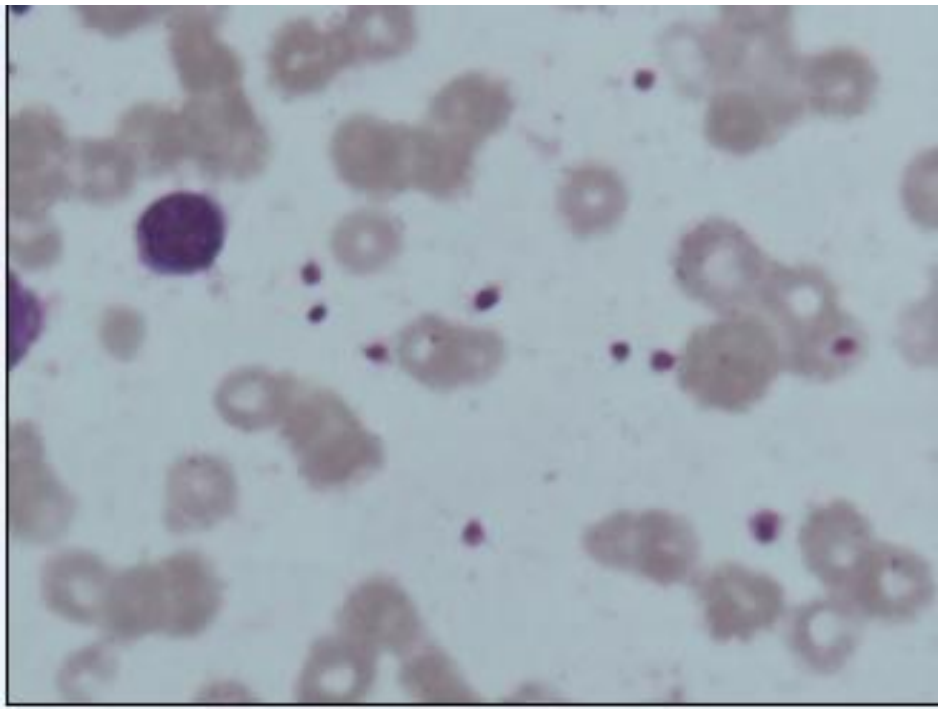


Figure F:PS showing microcytic anemia (patient: serial no 31)

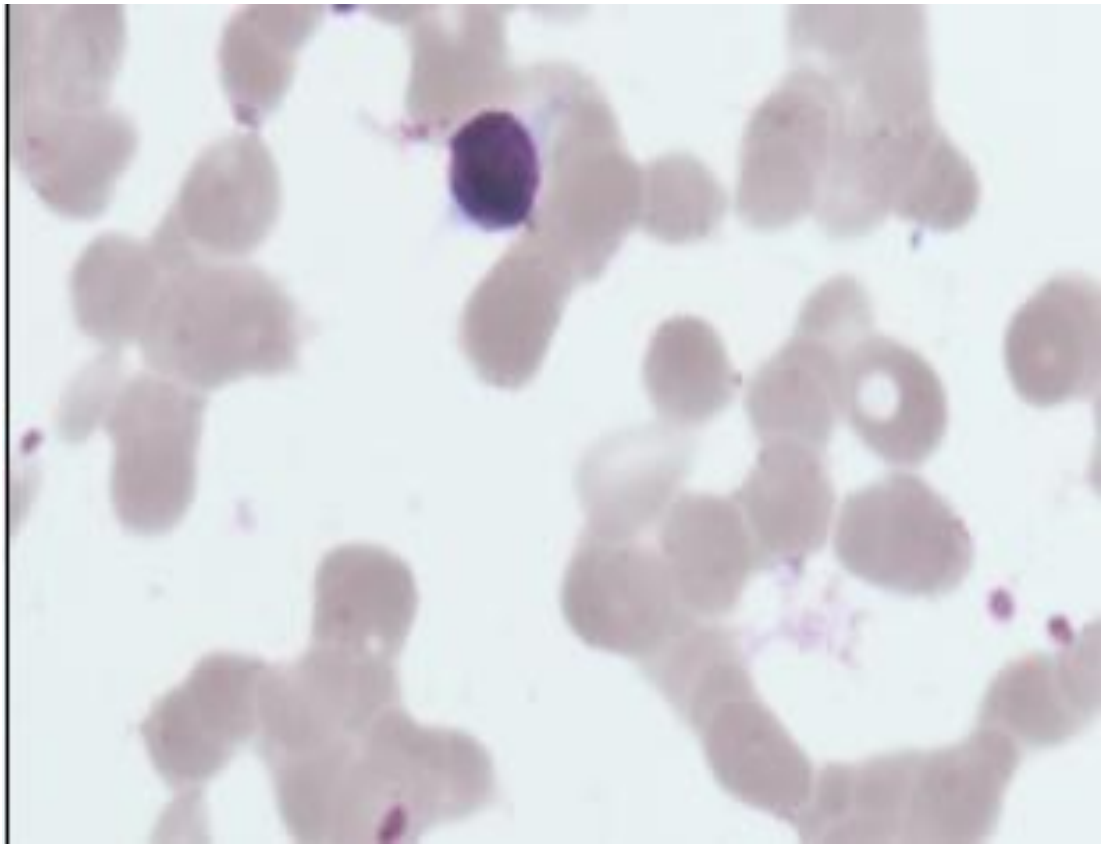


Figure G: PS of normocytic anemia(patient : Sl no 46)

Discussion:

Anemia is reported to be a common hematological abnormality in cancer [6]. The same was seen in our study. Anemia was observed in 72% of newly diagnosed patients with NHL. The frequency reported in our study is much higher compared to that of other studies reported in the literature. This may be due to the fact that the cut off level of hemoglobin for the diagnosis of anemia was much higher in our study (13g/dl and 12g/dl in men and women respectively as per WHO definition) whereas most of the studies reporting a lower frequency of anemia defined anemia as a hemoglobin level less than 11-12g/dl[7,72,64]. Moullet has also mentioned that studies reporting lower frequency have used lower hemoglobin levels to define anemia [58,76,77]

Studies	**Frequency of Anemia**	**Hb level used to define anemia(g/dl)**
Moullet et al(7)	32%	</=12
Morrow et al(64)	35%	<12
Ghosh et al(72)	42.41%	<11
Current study	72% 62.5%	<13males/< 12 female if cut off <11 is considered

The higher frequency may also reflect a higher prevalence of anemia in the Indian population compared to the West[2]. It is noteworthy that Ghosh et

al from Delhi, India, reported a frequency of 42%, which was higher than the other studies even though the level of Hb used to define anemia was lower than most of the western studies [72].In our study, the frequency of anemia was 62.5% (125/200) even if we consider a cut-off Hb level of <11g/dl to define anemia, which is, still higher compared to other studies.

Morrow et al reported a significant association of anemia with female sex.[64] We, however, did not find a significant association of anemia with female sex. The most common type of anemia reported in our study was anemia of chronic disease (ACD) and it was observed in 49% of patients. This is in concordance with most of the studies. Ghosh et al and Dubravka et al also observed that ACD was the most common type of anemia in patients with NHL. They reported ACD in 71% and 53.8% of the patients respectively.[72,63] However, the frequency of AIHA was less in our study compared to others. The frequency of nutritional anemia was also lower in our study population.

Studies	ACD(%)	Multifacorial%	Nutritional(%)	AIHA(%)
Ghoshl[72]	71.74	39.13	47.83	10.87
Our study	48.6	24.3	25.5	1.3

Anemia of chronic disease is a commonly reported phenomenon in cancer [8] as well as in Hodgkin lymphoma and is probably related to cytokine release[60,61,62]. This is probably also the cause of anemia in most cases of

NHL. This is reflected by the elevated CRP values reported in all patients with ACD in our study. [78]

Anemia was observed in 53 % of patients aged more than 60 years. On univariate analysis, we found a significant association of anemia with age more than 60 years. A similar finding is reported in other studies [7,59,64].

Studies	**% of patients >60years**	**p value for association with anemia**
Morrow et al[64]	61.4	0.0416
Moullet et al[7]	39	<0.001
Current study	53	0.02

The association of age with prognosis in NHL is well known and is included in the IPI [45,46]. It is, therefore, noteworthy that anemia is significantly associated with age> 60 in most studies. The cause of anemia in elderly is multi-factorial. It has been reported that anemia is common in elderly population by some studies [23] .On the contrary, other studies have refuted this.[24]. However, age seems to have a significant association with anemia as well as with attainment of CR after treatment.

Our study did not find a significant association between anemia and the presence of B symptoms. There was also no significant association between anemia and elevated LDH. This is in contrast to certain studies that have reported significant association of anemia with B symptoms as well as with elevated LDH [7,64]

Studies	B symptoms in anemia (%)	P value	Elevated LDH in anemia (%)	P value
Moulletl[7]	19	<0.0001	47	<0.0001
Morrow [64]	51.4	<0.0001	52	0.001
Current study	26	0.47	90	0.83

We found a much higher percentage of patients having elevated LDH. This may be due to a larger number of patients with high-grade lymphomas included in our study.

Our study did not find a significant association of anemia with any particular histological subtype of NHL.

There is a lack of consistency in literature regarding the relationship between anemia and histological subtypes of NHL. This may be because of the fact that various studies had a different distribution of patients with different grades of NHL. Moullet et al observed that the incidence of anemia was lower in indolent lymphomas (17% in follicular and 19% in marginal zone lymphomas) except in small lymphocytic or lymphoplasmacytoid (37%) lymphoma.[7] Conlan et al observed that patients having high-grade lymphoma had a lower median hemoglobin value and a higher prevalence of anemia than patients with intermediate or low-grade lymphomas[65]. On the other hand, Bloomfield et al did not find a significant difference among histology types as regards to the incidence of anemia [66]. Morrow et al observed that the

prevalence of anemia varied with histology subtypes. [64] Moullet et al suggested that increased cytokine production may be responsible for the variation in anemia patterns based on histology subgroups. [7] This study observed that a larger percentage of anemic patients had the following histology subtypes: large cell immunoblastic and large cleaved or noncleaved cell .

Our study found a significant association between bone marrow involvement and anemia (p=0.02). All patients (n= 13) with bone marrow involvement were found to have anemia. This is in concordance with some other studies. There are conflicting reports in literature regarding bone marrow involvement and anemia in lymphoid malignancies. Both Moullet et al and Bloomfield et al observed that bone marrow involvement was significantly associated with anemia ($p<0.0001$) [7,66]. But others have reported that although bone marrow involvement was associated with anemia, it was not statistically significant [65, 67]

Studies	**Moullet [7]**	**Bloomfield [66]**	**Conlan [65]**	**Stein [67]**	**Currentstudy**
association BM + with anemia	yes	yes	no	no	Yes

We found a significant association of anemia with complete response and complete /partial response in patients with NHL. We observed a statistically significant association of anemia as well as age with complete response in univariate analyses. Age more than 60 years was significantly associated with both anemia as well as lesser response to treatment. Hence, a multi factorial logistic regression analysis was done to determine whether anemia was independently associated with response to treatment irrespective of age. It was observed that both age more than 60 years and presence of anemia were independently and significantly associated with a higher risk of not attaining complete response (9 fold and 17 fold higher risk respectively). Age > 60 years is included in the IPI score and is a well known prognostic factor. However, the association of anemia with response to treatment, irrespective of age, is an interesting finding in this study. Several studies have reported on the prognostic significance of anemia in NHL. Gallagher reported that anemic patients had a lower incidence of CR compared to non anemic patients with DLBCL treated with R-CHOP chemotherapy (59% vs. 81%). Anemic patients also had a higher mortality rate, (37% vs. 16%), shorter median PFS [18 (0.7-52+) vs. 30 (2-57+) mos.], and shorter median OS [17 (0.8-59+) vs. 34 (5-60+) mos.]. In this study, 15 (56%) of the 27 anemic patients received erythropoietin stimulating agents (ESA).and they observed that, anemic patients who responded to ESA treatment (4/15) had a higher rate of CR (3/4 vs. 4/11)[71]. This is an interesting finding as erythropoietin can

then serve as an important treatment for patients with NHL having anemia. However, one should remember the negative impact of ESA on thromboembolic events and survival, especially when hemoglobin is raised above 12 g/dl in chemotherapy induced anemia. Randomized controlled trials are required to establish the role of ESA in such settings.

Other studies have also reported that anemia before chemotherapy or radiotherapy can have a negative correlation with survival[7,57,65,68,69,80] and also decreases response to therapy[59,64,70]. The impact of anemia on Follicular lymphoma is well known and is included in the FLIPI [11]. Gallager and Romaguera have highlighted the prognostic significance of anemia in this subset of patients [57,79]. There is limited data in the literature on the impact of pretreatment anemia on response to chemotherapy. Cowan et al analyzed the impact of anemia on the outcome of patients with high and intermediate grade NHL and found a negative correlation with survival [68]. Bremnes reported decreased response to treatment in anemic patients in patients with high grade lymphoma in Norway [70] Stewart et al also found a negative correlation of anemia with survival in patients with low grade lymphoma treated with chemotherapy.[80] Morel et al evaluated the factors that influence complete response in patients with NHL. He found that age >40 years, elevated LDH level, a low hemoglobin level (<10 g/dl), and presence of two or more extra nodal site involvement were associated with a poor response. But complete remission was measured over a longer period after chemotherapy. More over,

these patients were treated with four different chemotherapy regimens [59]. This is similar to our study where patients received different chemotherapy regimens depending on the subtype of NHL. This was one of the limitations in the study. On the other hand, Morrow et al exclusively included patients with IGNHL treated with CHOP and uniformity in chemotherapy regimen was maintained. Response to chemotherapy was inversely associated with baseline hemoglobin. They concluded that baseline anemia in IGNHL should be identified and appropriately managed. They analyzed the outcome of 546 patients who were treated with CHOP chemotherapy from 1991 to 1999. Anemic patients (Hb <12 g/dl) had a higher percentage of no response /progression than patients without anemia. Anemia at baseline was diagnosed in 46.1% to 51.7% of patients in the no response/progression group, as compared with 35% baseline anemia overall [64]

Moullet et al studied 1077 patients with NHL and reported on the frequency and prognostic significance of anemia. They found that the overall survival (OS) and progression-free survival (PFS) were significantly shorter for patients who were anemic at diagnosis in an univariate analysis. Median survival was, respectively, 47 and 146 months for overall survival and 15 and 64 months for progression-free survival depending on the presence and absence of anemia. .They also observed that anemia was significantly associated with a shorter overall survival in certain histological types of NHL such as Small Lymphocytic lymphoma and Lymphoplasmacytic lymphoma.

They also did a multivariate analysis ,crossing anemia with significant variables for overall and progression-free survival ,and found that anemia was an independent adverse prognostic factor for overall survival The inclusion of hemoglobin value to the five variables of the International Prognostic Index was found to improve the predictive value of IPI for OS and PFS .[7] Dubravka et al have ,in fact , mentioned that anemia was not included as a part of the International Prognostic Index (IPI) because it was not tested when the index was developed[63]

Studies	Impact of anemia on response to treatment/ survival
Moullet (7)	Yes
Morrow(64)	Yes
Gallagher(79)	Yes
Cowan(68)	Yes
Dixon(69)	Yes
Conlan(65)	Yes
Romaguera(57)	Yes
Stewart(80)	Yes
Dubravka(63)	Yes
Current study	Yes

Hence, we can summarize that various studies have shown a significant impact of anemia on response to treatment as well as on survival. However,

most of these studies were retrospective and the number of patients included was relatively small. Our study is a prospective study and the impact of anemia on response to treatment is significant and is independent of age. More such studies that include a larger number of patients and incorporate multivariate analyses to exclude confounding factors such as age and stage are needed to make definite conclusions about the impact of anemia on the prognosis of NHL.

Our study did not find any significant association between anemia and chemotherapy related toxicity. More over, there was no significant worsening of anemia after chemotherapy. This is in concordance with the study on anemia in lymphoid malignancies by Dubravka et al ,who concluded that chemotherapy in the treatment of lympho-proliferative disease does not worsen anemia, and anemia is mostly a sign of the disease severity.[63]On the contrary, some studies have found age to be a definite independent risk factor for neutropenia in patients older than 60 years with lymphoma in a number of prospective clinical trials of CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or regimens with equivalent toxicity [81,82,83,84] .

Conclusion:

Anemia is a common hematological abnormality in non-Hodgkin lymphoma. There are very few studies reporting on the frequency, distribution and prognostic impact of anemia on NHL. Our study was conducted with a view to throw some light on these aspects. The following conclusions may be drawn from this study:

- The frequency of anemia may be higher in NHL patients in India compared to western countries as observed in our study.
- Most common pattern of anemia was normocytic normochromic anemia. Median Hb was 10.4g/dl.
- Anemia in NHL is multi factorial. The most common cause of anemia was anemia of chronic disease .This may be related to inflammation and cytokine release in the background of malignancy as reflected by the elevated CRP in these cases.
- Median age of the study population was 59 years; Age > 60 years was significantly associated with anemia
- There was no significant association of anemia with female sex
- There was no significant association of anemia with specific subtype of NHL, B symptoms or elevated LDH
- There was significant association of presence of anemia with bone marrow involvement.

- There was significant association of anemia with attainment of response to treatment both on univariate and multivariate analysis, independent of age.
- There was no significant association of anemia with chemotherapy related toxicity and there was no significant worsening of anemia after treatment.

Limitations:

One major limitation of the study was the heterogeneity of the study population. Patients with different subtypes of NHL treated with different chemotherapy regimen were included and impact of anemia on the outcome of a particular subtype could not be assessed. Also, the toxicity profile of different chemotherapy regimens is likely to be different. Another limitation was the short period of study, as a result of which, the impact of anemia on disease free survival and overall survival was not analyzed. One of the other drawbacks is that age was the only factor included in the multivariate analysis to assess the association of anemia on CR. This is because, we found a significant association of age with CR as well as with anemia and it is a well known prognostic factor in NHL. However, other factors such as stage and sex are also important. More over, a larger number of patients is needed to draw more definite conclusions in this regard.

Anemia may have an independent prognostic impact on the outcome of patients with NHL. This study highlights the need for further prospective

studies, including larger number of patients, to establish such association, if any. This study is relevant in the Indian context as the prevalence of anemia is much higher in India compared to western countries[85]. In our study, we found a high frequency of anemia even when a cut off level of 11g/dl was considered to define anemia prompting evaluation, as per the National Comprehensive Cancer Network (NCCN) guidelines[86].

Anemia may also be correctable in most cases and the negative impact of anemia on response or survival may be overcome by timely intervention. Hence, it is extremely important to investigate the causes of anemia in any patient with malignancy It is equally essential to explore the possibility of anemia as an independent prognostic factor in NHL in future prospective studies. This can have a major impact on the overall management of patients with non-Hodgkin lymphoma.

Proforma

Name: age: sex:

UHID number: Index number: Date of presentation:

Diagnosis:		
Subtype:	Stage:	IPI:
Extranodal sites:	Y/N	
B symptoms:	Y/N	
Bone marrow involved:	Y/N	

COMPLETE HEMOGRAM

Total WBC count :	Hb:	Platelet Count:	DC:
MCV :	MCH:	MCHC:	Hct:
Reticulocyte count:	reticulocyte index:≥2.5/ <2.5		

Peripheral smear: Normocytic/Microcytic/Macrocytic

IRON INDICES:

Serum iron:	Serum ferritin:	TIBC:	%saturation:

OTHER LAB INVESTIGATIONS

Serum Vitamin B12:	Serum folic acid:	Coombs test:+/-
Serum LDH:	CRP:	stool occult blood/ stool RE:

BONE MARROW STUDY

Morphology	Cellularity:
Iron stain: 1+/2+/3+/4	bone marrow biopsy: +/-

Type of anemia:

Chemotherapy regimen:

Blood transfusion given before chemotherapy : Y/N

Response after 4 cycles : CR/ PR/ Stable disease/ progression

CR after 4 cycles: Y/ N

Chemotherapy related toxicity : Y/ N

Type of toxicity: 1. 2. 3.

Grade of toxicity: 1/2/3/4 1/2/3/4 1/2/3/4

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