

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



Dr.Naghmana Mazher

**HEMATOLOGY SECTION,
DEPARTMENT OF PATHALOGY**

**POSTGRADUATE
MEDICAL INSTITUTE
LAHORE**

**β2-MICROGLOBULIN AND LACTATE
DEHYDROGENASE 2 ISOENZYME IN
NON-HODGKIN LYMPHOMA:**

**CLINICO-HEMATOLOGICAL
CORRELATION**

INTRODUCTION

- Lymphomas are defined as malignancies of lymphoreticular system. It is not one cancer but a name for a group of related cancers that arise when a lymphocyte becomes malignant.

- Lymphomas represent clonal malignancies in which the majority of the cells are frozen at a single stage of normal differentiation.

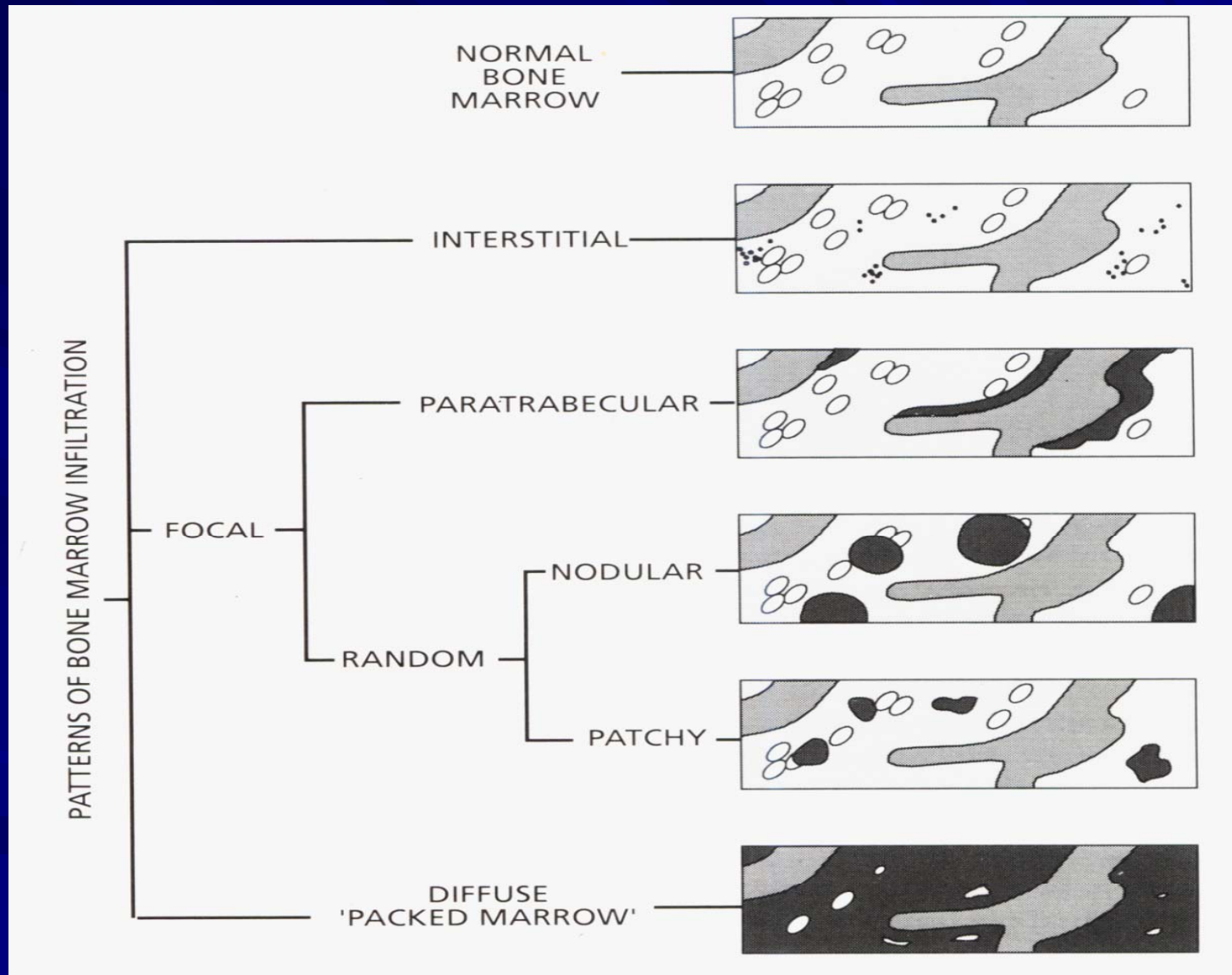
- Two broad types of lymphomas are Hodgkin disease (HD) and Non-Hodgkin lymphoma (NHL).

- About 85% of lymphomas are of B-cell origin and 15% of T cell origin. B-cells originate and mature in the bone marrow while T-cells also start out in the bone marrow but they differentiate and mature in the thymus gland.

- NHL constitutes an intimidating and extended family of lymphoid neoplasm encompassing diverse B-cell malignancies of lymph node follicle and several less common T-cell proliferations, plus a smattering of macrophage malignancies.

- Once histological diagnosis of malignant lymphoma has been established on lymph node biopsy, staging defines how widespread the disease is and locations of disease in the body.

PATTERNS OF BONE MARROW INFILTRATION IN NHL



- Patients at high risk for failure with conventional therapy may benefit from investigational approaches. Along with biological characteristics of tumor, laboratory data may also be helpful.

- The most important serological markers i.e. serum Beta-2-microglobulin (β 2m) reflects the tumor load and Lactate Dehydrogenase (LD) indicates proliferative activity along with invasive potential of lymphoma.

- LD catalyzes the reversible oxidation of lactate to pyruvate. It is expressed at higher levels when lymphocytes are dividing or when cells are distressed or damaged. Marked increase in its activity (upto 20 times the normal) is seen with lymphoma. Elevating LD is an indication of disease progression.

- It exists as five isoenzymes. These differ in catalytic, physical and immunological properties.

Nomenclature and Distribution of LD Isoenzymes

Nomenclature

Distributions

LD1	Red blood cells, heart, kidney
LD2	Red blood cells, heart, lymphocytes
LD3	Lung, spleen, pancreas, thyroid, adrenal
LD4	Kidney, liver, skeletal muscle, brain
LD5	Liver, skeletal muscle

- β_2m is a low molecular weight polypeptide, noncovalently linked to the heavy chain of class 1-histocompatibility antigen which are shed with cell turnover. It is plentiful on the surface of lymphocytes. Increased production or destruction of the cells cause β_2m levels in the blood to increase.

AIMS AND OBJECTIVES

- To determine the levels of serum lactate dehydrogenase2 (LD2) and β_2 microglobulin (β_2m) in patients of non-Hodgkin lymphoma (NHL).
- To correlate the levels of LD2 isoenzyme and β_2m in NHL patients with and without bone marrow infiltration.

MATERIAL & METHODS

STUDY PLAN

- **Study Design**

It was a cross sectional study.

- **Study Population**

The present study was conducted on 80 subjects irrespective of age and sex divided into following groups as follows.

Group A: Normal healthy controls
(n=20)

Group B: Patients of NHL without bone marrow infiltration
(n=30)

Group C: Patients of NHL with bone marrow infiltration
(n=30)

Study Universe

These cases were selected from following institutions;

- Lahore General Hospital, Lahore.
- Institute of Nuclear Medicine and Oncology (INMOL), Lahore.
- Services Hospital, Lahore.
- Mayo Hospital, Lahore.
- Jinnah Hospital, Lahore.

Inclusion Criteria

- Newly diagnosed cases of NHL by lymph node biopsy prior to the institution of chemotherapy of both sexes and all age groups were selected for the present study.

Exclusion Criteria

Patients with following conditions were excluded from the study.

- Myocardial infarction
- Renal failure
- Hepatic dysfunction
- Skeletal muscle disease
- Hemolytic anemia

- Malignancy of any other system
- Cerebrovascular accident
- Infectious mononucleosis
- Intestinal infarction
- Patients taking certain drugs like aspirin, clofibrate, fluorides and procainamide during the last one month.

Bone Marrow Examination

- Bone marrow aspiration biopsy
- Bone marrow clot and touch imprint
- Bone marrow trephine biopsy

Specialized Investigations

Serum β 2-microglobulin (β 2m)

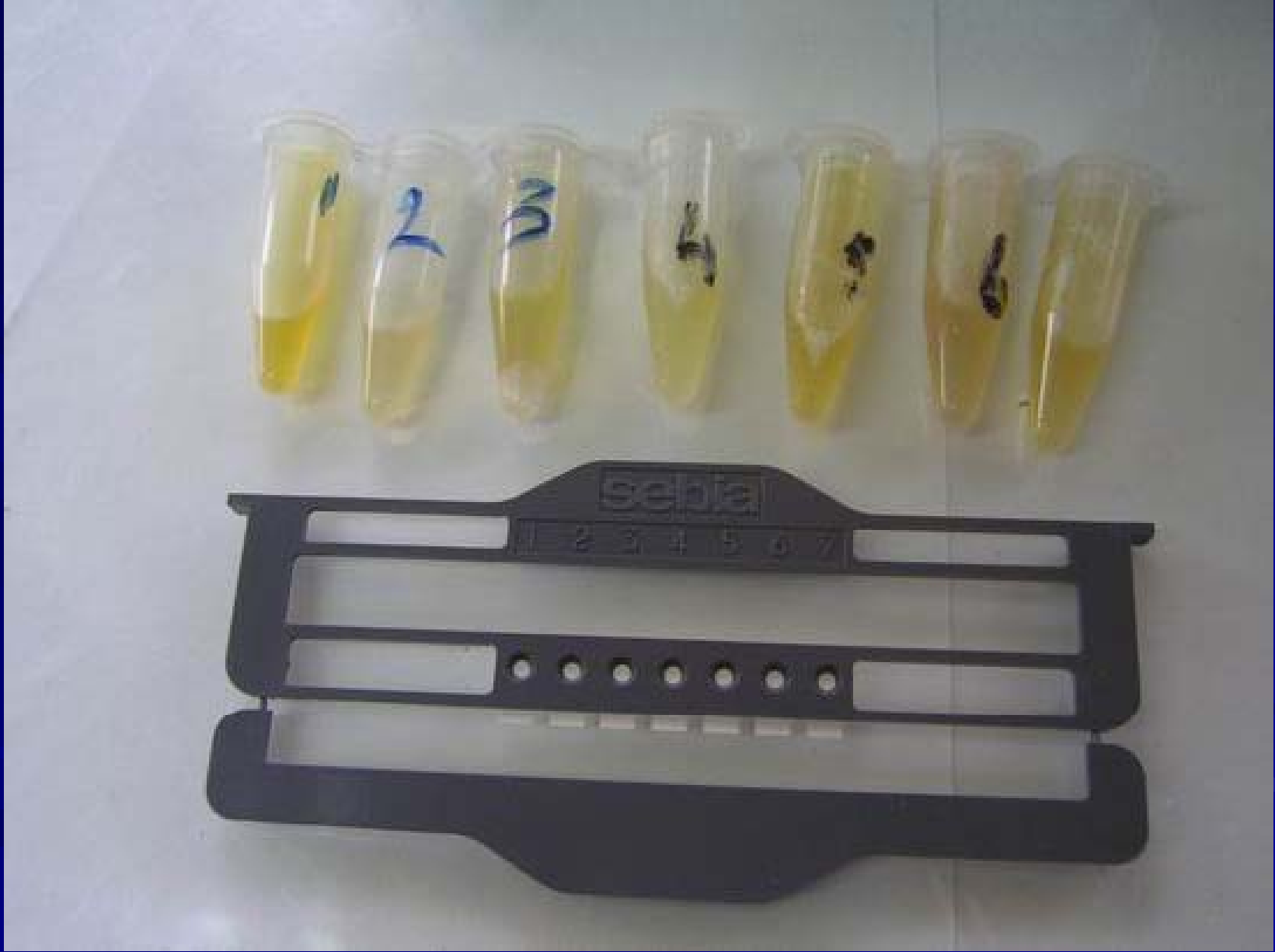
- It was done by ELISA (Enzyme linked immunosorbent assay)

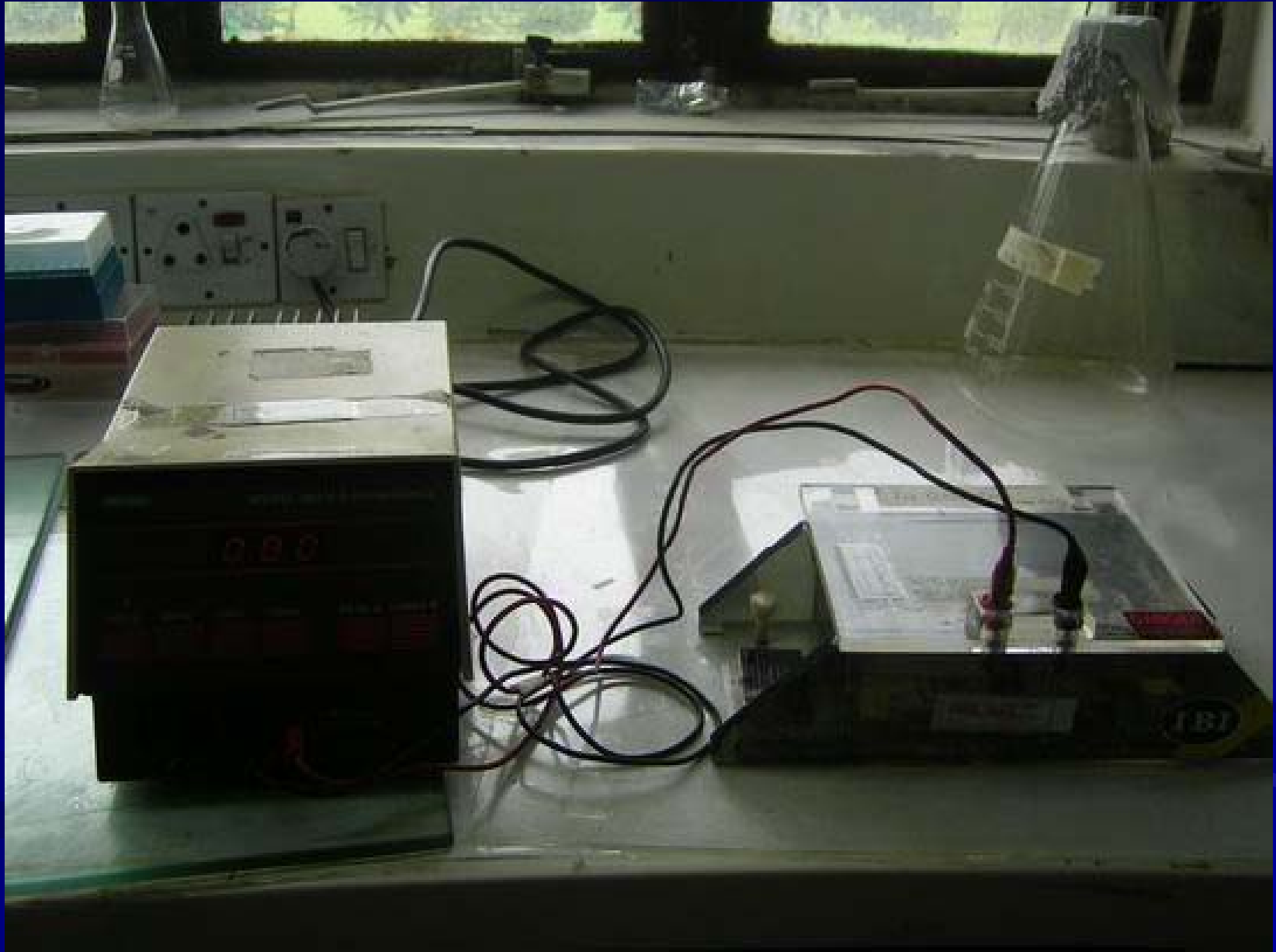
Serum lactate dehydrogenase 2 (LD2) Isoenzyme

- Serum LD2 isoenzyme level was measured by Agarose Gel Electrophoresis at Centre of Excellence in Molecular Biology (CEMB), Lahore.











**Photomicrograph No. 3.4
AGAROSE GEL SHOWING RAISED LD2
LEVELS AFTER ELECTROPHORESIS**

Results & Observations

Table 1 CLINICAL FEATURES OF NON-HODGKIN LYMPHOMA
(n = 60)

CLINICAL FEATURES	TOTAL	PERCENTAGE
Pallor	32	53
Fever	13	22
Night sweats	2	3
Weight loss	18	30
Lymphadenopathy	60	100
Splenomegaly	38	63
Hepatomegaly	22	36

Lymph Node Groups involved in NHL

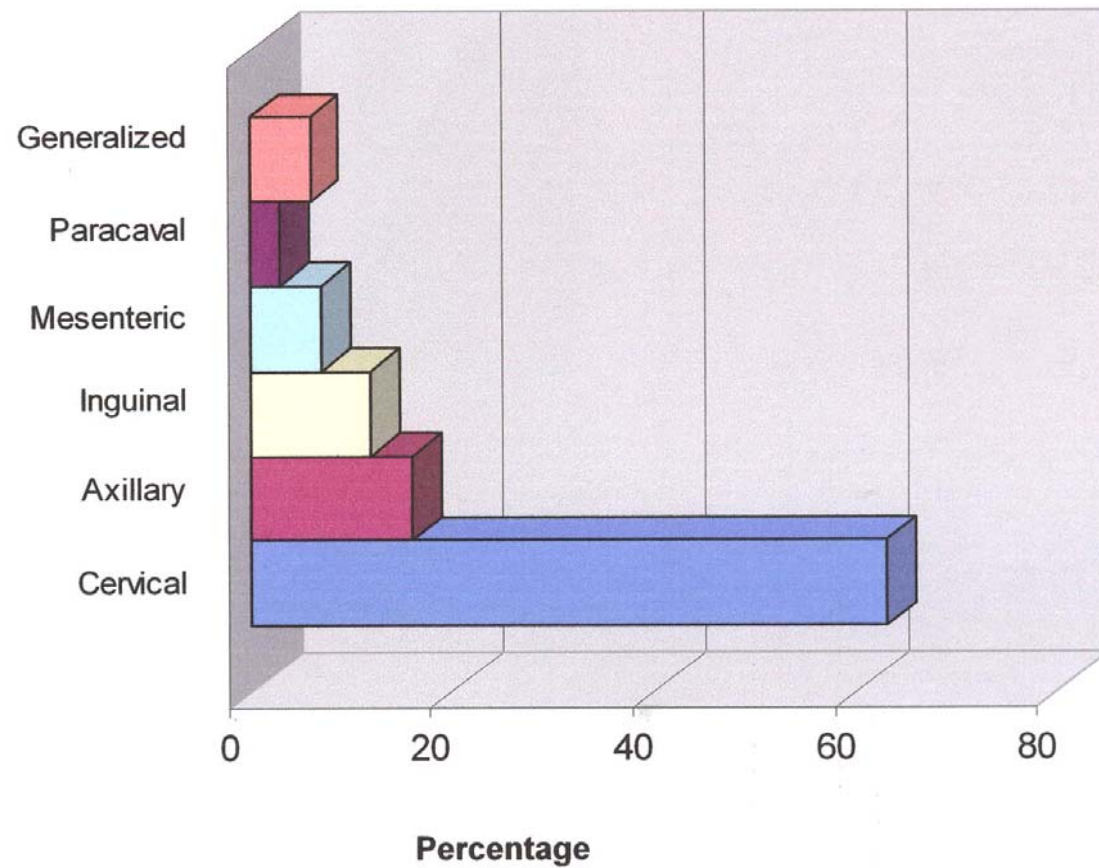


Table 2 FREQUENCY OF BONE MARROW INFILTRATION IN NHL PATIENTS – UNILATERAL VERSUS BILATERAL BONE MARROW EXAMINATION

TECHNIQUE	BONE MARROW INFILTRATION		TOTAL
	UNILATERAL	BILATERAL	
Bone Marrow aspirate	1 (3%)	3 (10%)	4
Bone Marrow clot	2 (7%)	5 (17%)	7
Bone Marrow imprint	10 (33%)	14 (47%)	24
Bone Marrow trephine	13 (43%)	17 (57%)	30

Note: Bone Marrow examination was done in 143 cases of NHL.

TABLE 3 PATTERNS OF INFILTRATION IN NON-HODGKIN LYMPHOMA

LYMPHOMA	DIFFUSE	INTERSTITIAL	PARATRABACULAR	RANDOM
NON HODGKIN'S LYMPHOMA n = 30	14 (46%)	10 (33%)	4 (13%)	2 (6%)
Mean β 2m	4.2 \pm 0.8	3.6 \pm 0.4	3.5 \pm 0.2	3.0 \pm 0.7
Mean LD2	55.07 \pm 2.97	52.1 \pm 4.9	48.5 \pm 5.06	45.5 \pm 6.3

n = Number of patients with bone marrow infiltration

Table 4

β2 MICROGLOBULIN AND LD2 LEVELS IN CONTROLS AND NHL PATIENTS WITHOUT BONE MARROW INFILTRATION

PARAMETERS	CONTROLS (n = 20)	NON INFILTRATION (n = 30)	P VALUE	NUMBER OF PATIENTS
β2M (μg/ml)	1.52 ± 0.43	2.41 ± 0.48	< 0.001 *	7 (12%)
LD2 (%)	29.1 ± 4.08	39.83 ± 2.09	< 0.001 *	6 (10%)

- Very highly significant

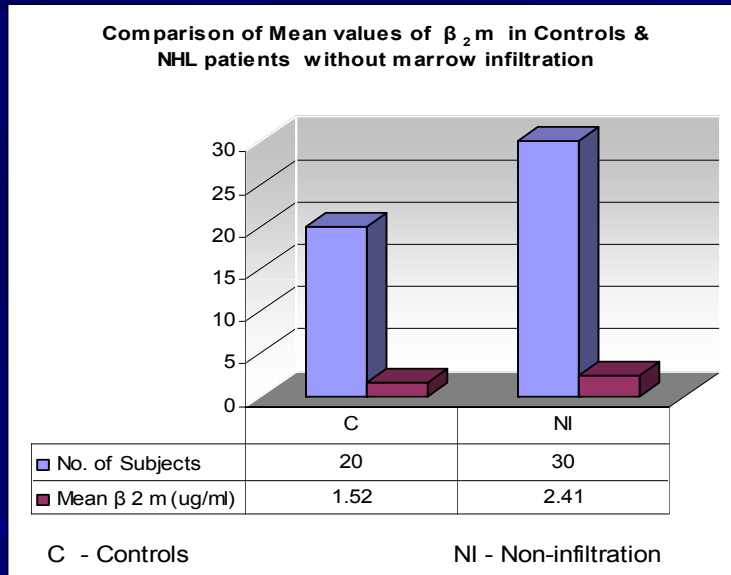


Fig 1 a:

Comparison of mean values of β₂ m in controls and NHL patients without marrow infiltration

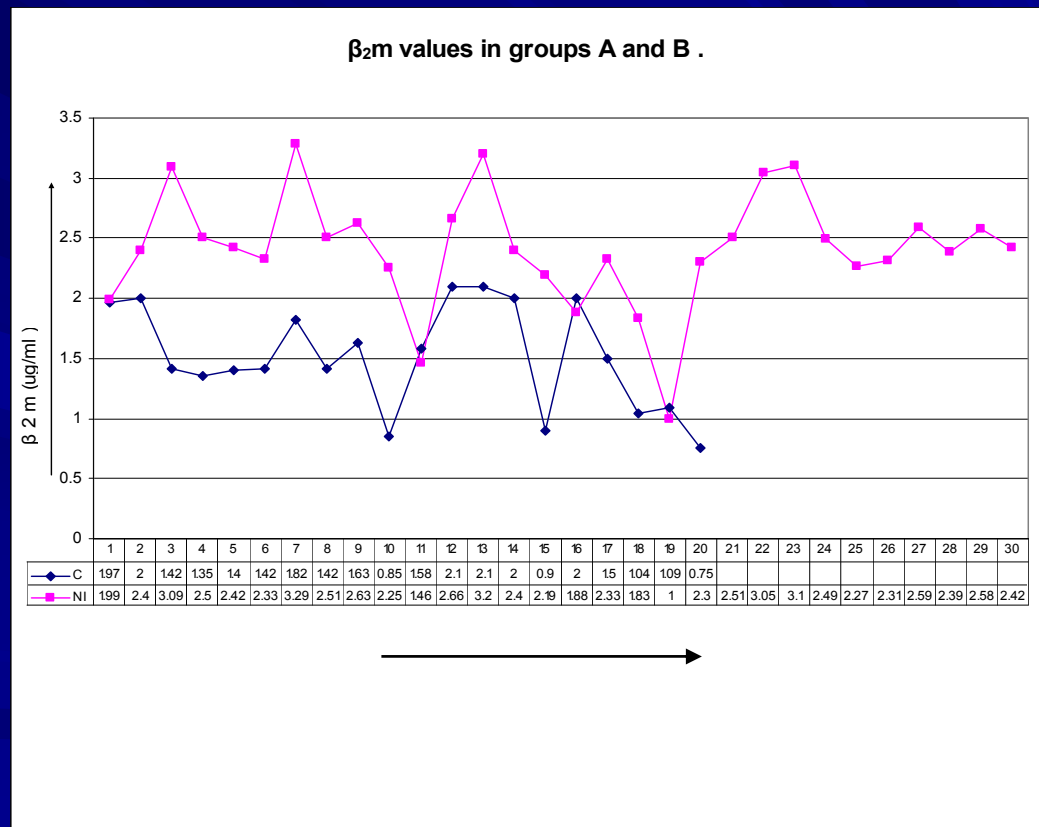


Fig. 1 b: β₂m values in groups A and B.

Table 5

β2 MICROGLOBULIN AND LD2 LEVELS IN CONTROLS AND NHL PATIENTS WITHOUT BONE MARROW INFILTRATION

PARAMETERS	CONTROLS (n = 20)	NON INFILTRATION (n = 30)	P VALUE	NUMBER OF PATIENTS
β2M (µg/ml)	1.52 ± 0.43	2.41 ± 0.48	< 0.001 *	7 (12%)
LD2 (%)	29.1 ± 4.08	39.83 ± 2.09	< 0.001 *	6 (10%)

- Very highly significant

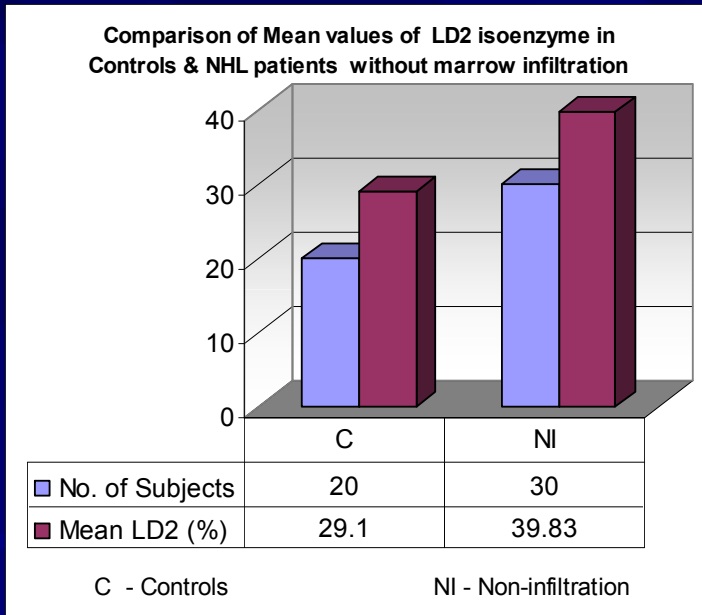


Fig 2 a

Comparison of mean values of LD2 isoenzyme in controls and NHL patients without marrow infiltration.

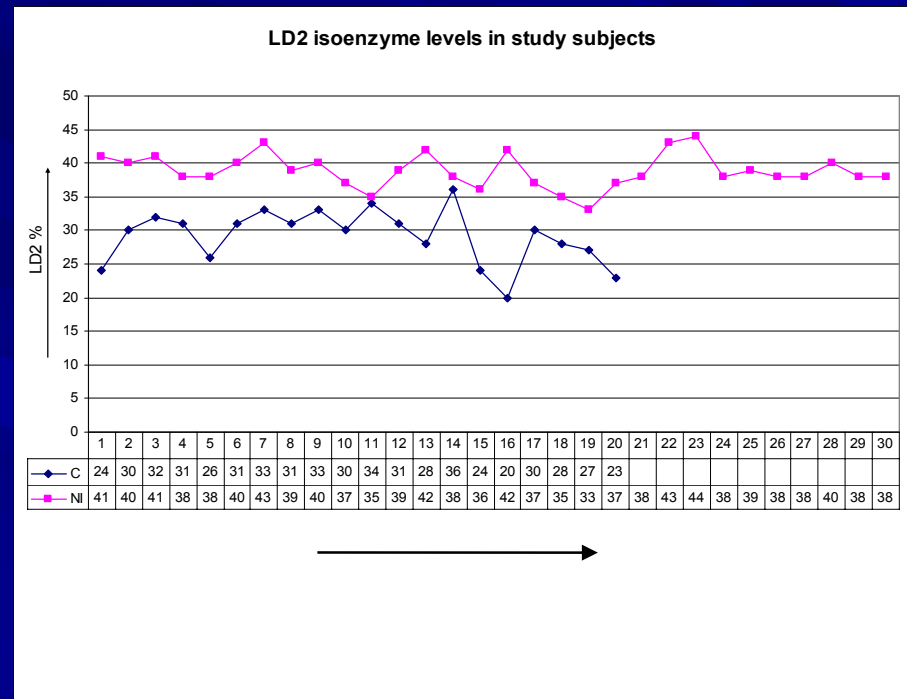


Fig 2 b: LD2 isoenzyme levels in study subjects.

Table 7

β2- MICROGLOBULIN AND LD2 LEVELS IN CONTROLS AND NHL PATIENTS WITH BONE MARROW INFILTRATION

PARAMETERS	CONTROLS (n = 20)	INFILTRATION (n = 30)	P VALUE	NUMBER OF PATIENTS
β2m (μg/ml)	1.52 ± 0.43	3.93 ± 0.71	<0.001*	29 (48%)
LD2 (%)	29.1 ± 4.08	52.53 ± 4.47	<0.001*	27 (45%)

* Very highly significant

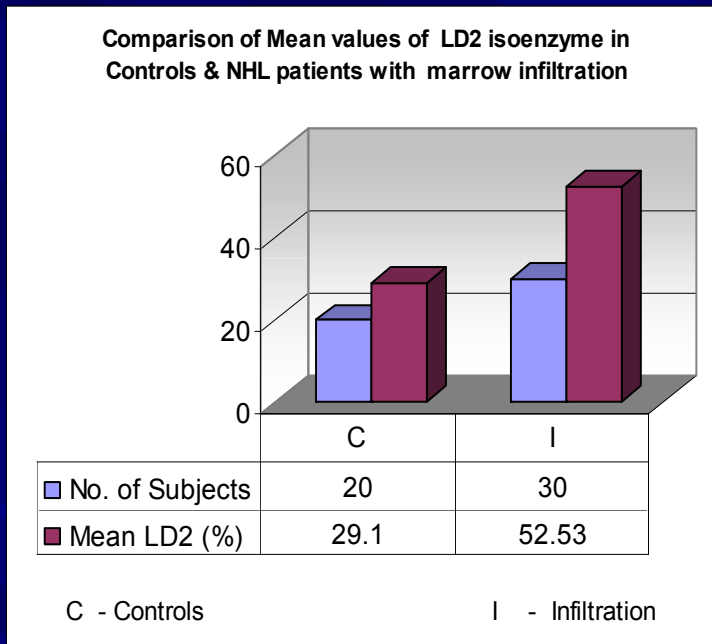


Fig 4 a: Comparison of mean values of LD2 in controls and NHL patients with marrow infiltration.

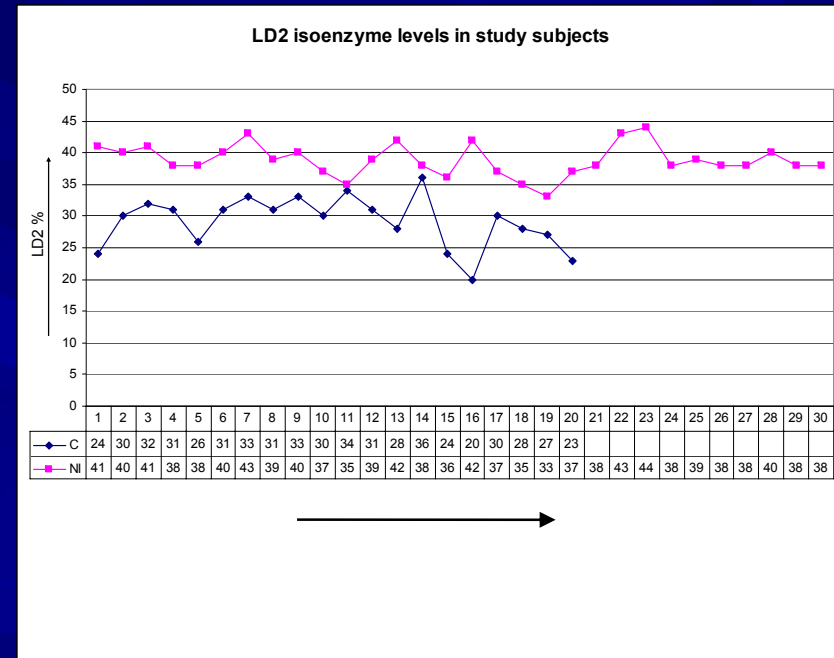


Fig 4 b: LD2 isoenzyme levels in study subjects.

Table 8

COMPARISON OF β_2m AND LD2 IN PATIENTS OF NON-HODGKIN LYMPHOMA

Parameters	Non infiltration (n = 30)	Infiltration (n = 30)	P Value	NUMBER OF PATIENTS	
				Non Infiltration	Infiltration
β_2m ($\mu g/ml$)	2.41 ± 0.48	3.93 ± 0.71	$<0.001^*$	7 (12%)	29 (48%)
LD2 (%)	38.83 ± 2.52	52.53 ± 4.47	$<0.001^*$	6 (10%)	27 (45%)

* Very highly significant

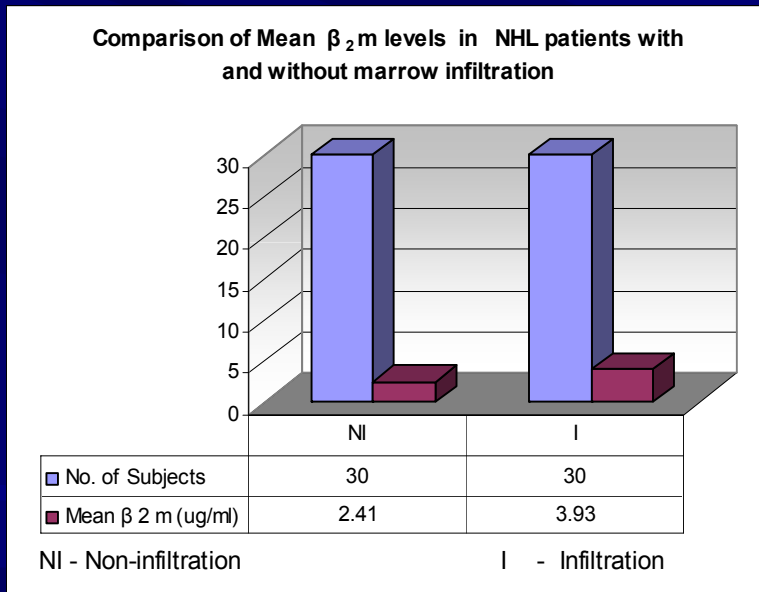


Fig 5 a: Comparison of mean β_2m levels in NHL patients with and without marrow infiltration.

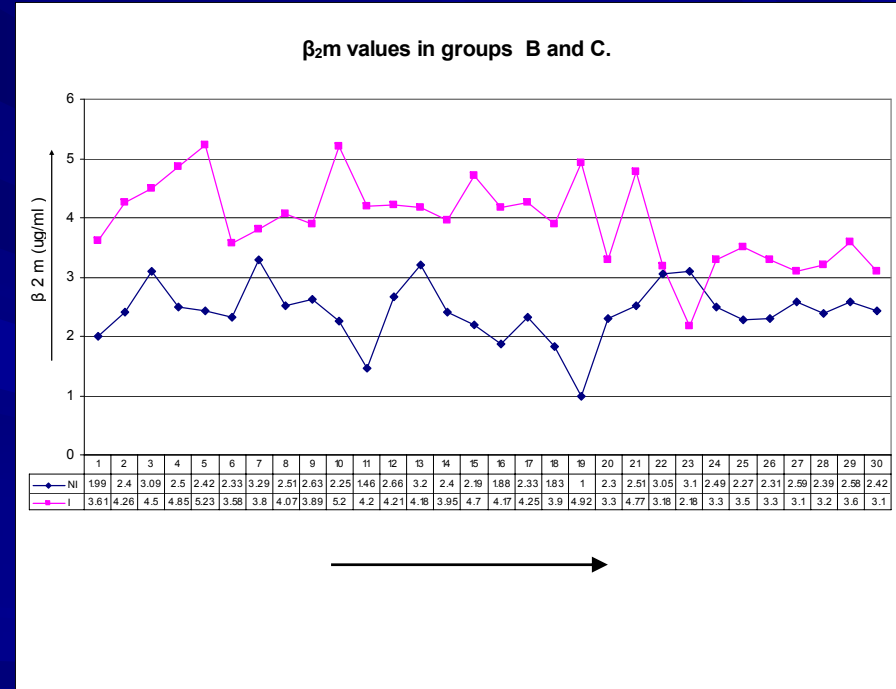


Fig 5 b: β_2m values in groups B and C.

Table 9

COMPARISON OF β 2m AND LD2 IN PATIENTS OF NON-HODGKIN LYMPHOMA

Parameters	Non infiltration (n = 30)	Infiltration (n = 30)	P Value	NUMBER OF PATIENTS	
				Non Infiltration	Infiltration
β 2m (μ g/ml)	2.41 \pm 0.48	3.93 \pm 0.71	<0.001*	7 (12%)	29 (48%)
LD2 (%)	38.83 \pm 2.52	52.53 \pm 4.47	<0.001*	6 (10%)	27 (45%)

* Very highly significant

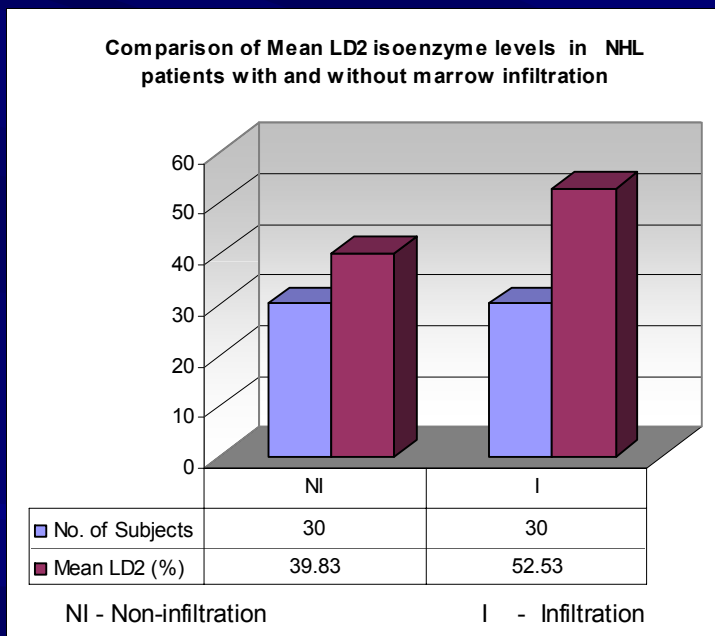


Fig 6 a: Comparison of mean LD2 isoenzyme levels in controls and NHL patients with and without marrow infiltration

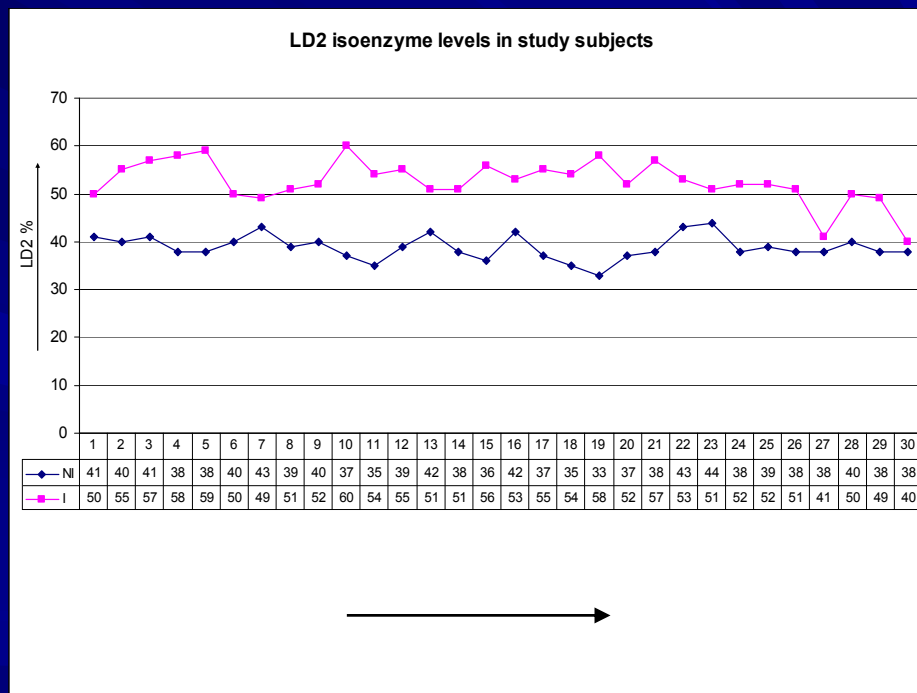


Fig 6 b: LD2 isoenzyme levels in study subjects.

Table 10 CORRELATION OF β 2m and LD2 ISOENZYME IN NHL PATIENTS GROUPS B AND C

	Group B	Group C
r – value	+ 0.73	+ 0.75
p – value	< 0.01 *	< 0.01 *

* Significant

Other Studies:

- Alici et al 2003
- Bairey et al 2003
- Ha et al 2003
- Bien et al 2004
- Blen et al 2004
- Boufia et al 2004
- Chen et al 2006
- Tong et al 2009

- Penz et al 2001
- Albitar et al 2007

CONCLUSION

- Diffuse pattern of infiltration was the most common pattern in bone marrow infiltration on trephine biopsy.

- By performing bilateral bone marrow biopsy examination, the percentage of positive cases with marrow infiltration was increased.

- Comparatively raised $\beta 2$ m levels were observed more in patients of NHL with bone marrow infiltration.

- LD2 isoenzyme values were elevated more in NHL patients with infiltration.

- So β_2 m and LD2 measurements can serve as indicator of extent of disease in NHL.

- Abnormalities in other hematological parameters were observed in cases with the involvement of bone marrow by the tumor and with peripheral seeding of lymphoma cells.

THANKS