Significance of Sudan Black-B Staining in Identifying Lymphoma Infiltration of Bone Marrow

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Background. In lymphoma infiltrations there in not only prominence of lymphocytes in the marrow but cells with an atypical morphology are also commonly seen. These lymphoma cells are difficult to differentiate morphologically from those of myeloid series. Cytochemcial staining has been used in diagnosis of acute leukemias for more than 20 years. However, value of Sudan Black-B (SBB) stain in the detection of lymphoma cells has not yet been elucidated. This stain differentiates lymphocytic proliferations from the non-lymphocytic ones.

Methods. Eighty cases of lymphoma diagnosed on lymph node biopsy were included in this study. Bone marrow aspiration, clot and trephine biopsy along with complete blood counts were performed in all these cases.

Results. Out of 25 case of Hodgkin Disease (HD), 22 cases (88 %) did not show evidence of infiltration when stained with Giemsa and 3 were positive for infiltration (12 %). SBB showed negatively stained atypical lymphocytes in 5 (20 %) cases whereas 20 (80 %) patients showed no such evidence in their marrow aspirates. The positive yield rose to 6 (24%) patients when their trephine biopsies were stained with H and E stain. Out of 55 case of Non- Hodgkin Lymphoma (NHL), 15 (27.3 %) showed infiltration on Giemsa stain, while on SBB stain atypical lymphocytes were noted in 20 (36.4 %) cases. Trephine biopsies of these patients showed infiltrates in 32 (58.2 %) cases.

Conclusion. Identification of positive cases becomes easier Sudan Black B Staining. It is as good as trephine biopsy. In cases where trephine biopsy is not possible, staining with SBB can be of additional diagnostic value for assessing extent of infiltration in both HD & NHL.

Key Words. Lymphoma, Bone marrow, sudan Black - B.

Introduction

Neoplastic proliferation of the cells of the lymphoid series can result into solid tissue tumors the malignant lymphomas. They present a heterogenous group of neoplastic disease, which vary in clinical presentation, histological types, course and response to therapy.

Ahmad et al[1] reported lymphomas as the commonest malignancy in adult males and 6th commonest in females in northern Pakistan. They also observed lymphoma along with leukemias as the most common malignancy in both sexes below 15 years of age.

The two major categories of malignant lymphoma are Hodgkins disease (HD) representing slightly less than half of all cases while non-Hodgkins lymphomas (NHL) make up the remainder.

HD is characterized by the presence of binucleate Reed Sternberg giant cells with prominent nucleoli and variable number of lymphocytes, plasma cells and eosinophils.

NHL is a more heterogeneous group of disorders with various histological types.

For staging in lymphoma patients, bone marrow biopsy is of substantial practical importance. Occasionally bone marrow is the only organ infiltrated and therefore a bone marrow biopsy is the prime diagnostic choice in lymphomas[2].

Cytochemcial staining has been used in diagnosis of acute leukemias for more than 20 years[3]. However value of Sudan Black-B (SBB) stain in the detectionof lymphoma cells has not yet been elucidated. This stain differentiates lymphocytic proliferations from the non-lymphocytic ones i.e, myeloid lineages.

SBB is essentially negative in lymphoid series cells. It has been observed that in lymphoma infiltrations there in not only prominence of lymphocytes in the marrow but cells with an atypical morphology are also commonly seen. In certain aspirations, these lymphoma cells are difficult to differentiate morphologically from myeloid series especially when the cytoplasmic granules are not stained. Lymphoma cells being of large size with atypical nuclear morphology are sometimes confused with myeloid precursors.

It has also been observed that cases with lymphoma infiltration in trephine biopsy invariably have atypical lymphocytes in their marrow aspirate. So the SBB staining may offer great help in lym-

Material and Methods

Eighty (80) cases of lymphoma diagnosed on lymph node biopsy were included in this study. Bone marrow aspiration, clot and trephine biopsy along with complete blood counts were performed in all these cases . These cases was referred from Inmol & Services Hospital.

Complete blood picture and platelet count were performed on an electronic counter.

Bone marrow aspiration was done from posterior iliac crest and trephine biopsy was taken. The marrow aspiration was performed by Islam's bone marrow aspiration needle from site 1-2 cm distant to the trephine biopsy. A septic measures were adopted during the procedure.

Bone marrow trephine was performed prior to bone marrow aspiration from posterior superior iliac spine by using Islam's bone marrow trephine needle having core securing device to prevent the biopsy slipping out of the needle during the process of extraction and obtain a uniform specimen without damaging the marrow architecture. Biopsy specimen was decalcified, block were prepared and stained with H & E.

Bone marrow smears were stained with Giemsa and Sudan Black'B (SBB). Histological section were made for bone marrow clot and trephine biopsy. The section were stained with Haemotoxyline and Eosin (H&E).

All the above preparations were examined under the microscope for the evidence of marrow infiltration.

Results

Hb:

Hb ranged from 7.8 -13.5 gm/dl with a mean (± SD) value of 10.7±1.7 gm/dl in non-infiltrated patients of HD and 7.1-12.4 gm/dl with a mean±(SD) value of 10.2±1.5 gm/dl in patients with infiltration. In NHL, Hb ranged from 7.9-13.6 gm/dl with an average of 10.8±1.7 gm/dl among those with infiltration and 8.6 to 14.1 gm/dl with an average of 11.2±1-5 gm/dl among those without infiltration. The differences were in-significant (P>0.05) when Hb levels of patients with and without marrow infiltration were compared, both in HD & NHD (Table 1&2).

TLC:

In HD, TLC ranged from 3.7-16.3×10^9/L, mean being 7.6±3.2×10^9/L, among those without infiltration and 3.6 -15.8×10^9/L (Mean SD)= 7.3±3.4×10^9/L). Only one case of TLC < 4.0×10^9/L was seen among patients without bone marrow infiltration.

In NHL, TLC ranged from 4.3-15.9×10^9/L with a mean (SD) of 8.7±2.6×10^9/L among those with infiltration while those without infiltration had a mean TLC of 8.1± 2.3×10^9/ L.

The differences between patients with or without bone marrow infiltration were non significant (P>.0.5) (Table 1&2).

ESR:

In HD, ESR ranged from 15-75 mm/Ist hr with an average value of 32±12.7 mm/Ist hr among those with no infiltration and 44 ± 13.4 mm/Ist hr. In NHL, the range was 20-80mm/1^{st} hr with an average of 42±20.5 mm/1^{st} hr among those without infiltration and 56±18.5 mm/hr among those with infiltration. The differences were insignificant when the values between with and without bone marrow infiltration were compared (P> 0.05) (Table 1&2).

Platelet Count:

In HD, patients platelet count ranged from 152-498×10^9/L with an average count of 328±84×10^9/L among those without infiltration and 150 - 412×10^9/L with an average value of 284±92×10^9/L among those with infiltration. Thrombocytosis (>500×10^9/L) was found in only one patient with out bone marrow infiltration. In NHL, the range was 84-476×10^9/L with an average of 216±98×10^9/L among those without infiltration and 198 ± 84×10^9/L among those with infiltration. Thrombocytopenia was present in two cases and both of them had evidence of marrow infiltration (Table 1&2).

Bone Marrow Aspiration:

Bone marrow aspirates, clot preparation, trephine biopsy and biopsy sections were examined to see lymphoma infiltration. The bone marrow aspirates were stained by Giemsa (GS) and Sudan Black-B (SBB) stain while bone marrow clot and trephine biopsy were stained by H & E staining.

A total number of 80 patients were referred to our department for staging by bone marrow biopsy. Out of these 25 (20%) patients were diagnosed cases of HD and 55 (68%) with NHL.

Out of 25 case of HD, 22 cases (88 %) did not show evidence of in filtration (table-III) when stained with Giemsa and 3 were positive for infiltration (1.5 %).

SBB showed negatively stained atypical lymphocytes in 5 (20 %) cases where as 20 (80 %) patients

Table 1: Comparison of Haematological Parameters in infiltrated and Non-infiltrated bone marrow in Hodgkins Disease (HD).

Para meters	Infiltration	No. Infiltration	P-Value
Hb. (gm/dl)	10.2 ± 1.5	10.7 ± 1.7	>0.05
TLC (10^9 /L)	7.3 ± 3.4	7.6 ± 3.2	>0.05
ESR. (mm/hr)	44 ± 13.4	32 ± 12.7	>0.05
Platelet count (10^9/L)	284 ± 92	328 ± 84	>0.05

Table 2: Comparison of Haematological Parameters in infiltrated and Non-infiltrated bone marrow in Non-Hodgkins Lymphoma (NHL).

Para meters	Infiltration	No. Infiltration	P-Value
Hb. (gm/dl)	10.8± 1.7	11.2± 1.5	>0.05
TLC (10^9/L)	8.1± 2.3	8.7 ± 2.6	>0.05
ESR. mm/hr	56±18.5	42± 20.5	>0.05
Platelet count (10^9/L)	198± 84	216± 98	>0.05

Table 3: Evidence of infiltration on Giemsa and Sudan Black B. staining in bone marrow aspirates of HD (n=25).

Stain	No evidence of BM infiltration	BM infiltration present
Giemsa	22	03
SBB	20	05
H & E	19	06

Table 4: Evidence of infiltration on Giemsa Sudan Black B. staining in bone marrow aspi-rates of NHL (n= 55)

Stain	No evidence of BM infiltration	BM infiltra-tion present
Giemsa	40	15
SBB	35	20
H&E	32	23

showed no such evidence in their marrow aspirates.

The positive yield rose to 6 (8.51%) patients when their trephine biopsy were stained with H and E stain.

Out of 55 diagnosed case of NHL 15 (27.3%) showed evidence of infiltration on (GS) stain, while on SBB stain atypical lymphocytes were noted in 20 (36.4%) cases. Histological section of trephine biopsies of these patients showed lymphoma infiltrates in 32 (58.2 %) cases (Table 3&4).

Discussion

Bone marrow aspiration and trephine biopsy are commonly performed in the staging of patients with newly diagnosed lymphomas[5-8]. However, bone marrow trephine is difficult to perform in children and obese patients. Of course, biopsy is compared with peripheral blood parameters like Hb, ESR, TLC,DLC and platelet count. These parameters are helpful in predicting marrow infiltration but reliable information can only be obtained by doing bone marrow biopsy.[9,10]

In the present study, Sudan Black-B stain applied to bone marrow aspirates proved quite useful in diagnosing marrow infiltrations by lymphoma cells.

Suspicious, atypical mononuclear cells seen with Giemsa stain in bone marrow smears belonged to lymphoid as well as myeloid series. In many cases granules in the myeloid series were not stained properly due to cellular abnormalities, staining defects (changes) and other inherent errors[11]. This problem was overcome by applying Sudan Black-B stain to bone marrow smears of lymphoma, the lineage of these cells (lymphcytic)[12]. To bone marrow

infiltration by assessing the number of negatively stained cells[13]. Our study showed that identification of positive cases becomes easier and better results are obtained with Sudan Black B Staining. It is as good as trephine biopsy. In cases were trephine biopsy is not possible and for young haematologist staining with SBB can be of additional diagnostic value for assessing extent of infiltration in both HD & NHL.

We conclude the SBB stain should be applied routinely to all the bone marrow aspirate of lymphoma patients.

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