Original Research Article

Clinico-pathological and Immunophenotype Analysis of Hairy Cell Leukemia Cases – A Single Institute Experience

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Abstract

Background: Hairy cell leukemia is a mature lymphoid B cell disorder, characterized by hairy cells, a specific genetic profile, different clinical course and the need for an appropriate treatment. It is seen primarily in elderly, characterized by a triad of splenomegaly, pancytopenia and monocytopenia.
Aim: To evaluate clinicopathologically and with immunophenotyping hairy cell leukemia cases received at our institute in conjunction with similar studies.
Material and methods: This is a retrospective study which included 7 cases over a period of 3 years (2019-2021) confirmed on morphology and flow cytometry.
Results: The study revealed 7 cases which showed patients with age ranging from 34-65 years. M:F ratio was 6:1. Two cases were covid positive (28.5%). Most of the cases presented with fever, weakness (28.5%). Splenomegaly was seen in three of the cases (42.6%). Laboratory investigations revealed anemia in 71% cases, leucopenia in 56.8%, lymphocytic prominence in 100% and pancytopenia in 14.2%. One patient presented with leukocystosis (14.2%). Marrow was hemodiluted and aparticulate in 3(42.6%) cases. Hairy cells were seen on morphology of peripheral smear and marrow aspirate. On flow cytometry, CD5 negative in all cases (100%), CD10 positive in 2(28.5%) and CD23 in 2 cases (28.5%). Few cases confirmed BRAF v600e mutations.
Conclusion: Unusual findings like leukocytosis, absence of spleen, presence of lymphadenopathy can be present in hairy cell leukemia. Classical fried egg appearance in trephine biopsy may not be a

feature in all the cases. CD123 is expressed in covid patients unlike other studies and further research is needed to establish the loss of CD123 in covid patients.

Key words

Hairy cell leukemia, Flow cytometry, Immunophenotyping.

Introduction

Hairy cell leukemia is a mature lymphoid B cell disorder, characterized by hairy cells, a specific genetic profile, different clinical course and the need for an appropriate treatment. It is seen primarily in elderly, characterized by a triad of splenomegaly, pancytopenia and monocytopenia [1]. Hairy cell leukemia is a cytologically and immunophenotypically distinct rare indolent small mature lymphoid cells with hairy projections involving peripheral blood, marrow and spleen [2]. Splenomegaly is a prominent feature ranging from 70-100% of the cases. Absence of splenomegaly and dry tap of marrow misguides a case of HCl towards aplastic anemia [3].

Immunophenotyping studies like flow cytometry and IHC can confirm Hairy cell leukemia. Leukemic cases express CD20, CD22, CD11c, CD25, CD103, CD123, FMC7, CD200 and cyclin D1. Most cases lack CD5, CD10. However, CD 10 is seen in 10-20% cases. Annexin A1 and CD123 are markers used to distinguish HCL from Splenic marginal zone lymphoma and Hairy cell leukemia variant [4].
Three or four CD markers are helpful in the diagnosis of Hairy cell leukemia in most of the cases. CD 123 expression is specific for Hairy cell leukemia that distinguishes from Hairy cell leukemia variant [5].

Materials and methods

This is a retrospective study of 3 years 2019-2021.Hairy cell leukemia cases were further evaluated due to suspicion of hairy cell on morphology and diagnosis confirmed on flow cytometry.

Peripheral blood sample or bone marrow aspiration specimens were used in flow cytometric analysis. All the cases were consistent with Hairy cell leukemia.

Flow cytometry

Peripheral blood or bone marrow aspirate in EDTA tubes were processed for immunophenotyping. The cells were lysed, washed and stained with antibodies tagged with various fluorochromes. Eight color flowcytometry was performed .Cells were stained with various combinations of flouorochromes like FITC (Flourocein isothiocyanate), PE (Phycoerythrin), Peridinin chlorophyll protein (Per cp), Per cp-cy5.5, allophycocyanin (APC), Apc-H7, V-450, V-500 labeled monoclonal antibodies.

The monoclonal antibodies used were CD19, CD5, CD10, CD 23, CD200, CD 20, CD43, CD79b, Anti-kappa, Anti-lambda, CD22, CD38, FMC-7, CD103, CD11c, CD25 and CD 123.

Cells were incubated in dark for 30 minutes at room temperature and washed with phosphate buffered saline. Data analyzed using FACS DIVA software. Cells gated on lymphoid and monocytoid region. Percentage of hairy cells were ranging from 15 –70%.

Flow cytometry analysis was done on either peripheral blood sample or marrow aspirate depending on the availability of the samples as in some of the cases marrow was hemodiluted and aparticulate. All the cases were consistent with Hairy cell leukemia.

Treatment details and response to treatment could not be traced in majority of the cases. One case showed morphological remission on bone marrow aspiration done after 6 months of treatment.

Results

Age of patients ranged from 34-65 years with median age of 52 years. All the patients were males except one case with male to female ratio of 6:1. Most common complaints were fever and weakness. Physical examination revealed splenomegaly in 3 of the cases. Laboratory investigations revealed anemia in five cases, with hemoglobin ranging between 7-14 gm/dl. Total leukocyte count was normal in four cases, leucopenia in two cases, monocytosis in three cases and leukocytosis in one case which is very rare. Thrombocytopenia was seen in one case, pancytopenia in one case, hemodiluted and aparticulate marrow in three cases. Covid positivity was in two cases. Bio-chemical investigations showed HbsAg positivity in one case (**Figure – 1 to 3, Table – 1 to 3**).

Figure – 1: On morphology classic hairy cells with hairy cytoplasmic projections and nucleus with smooth contour (Leishman stain, 100X).

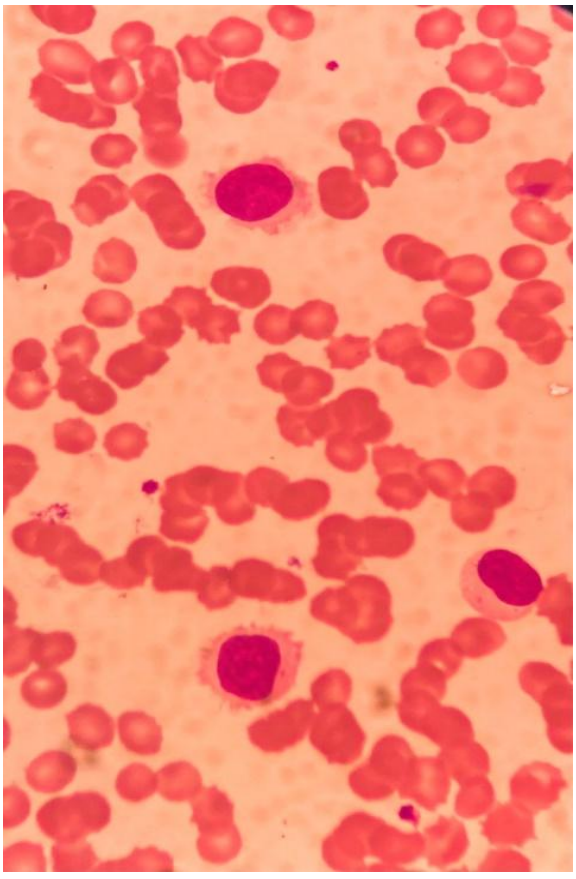

Figure - 2: On morphology classic hairy cells with hairy cytoplasmic projections and nucleus with smooth contour (Leishman stain, 1000X).

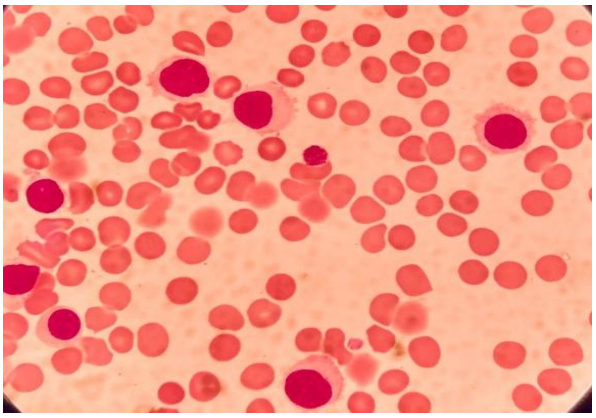

Figure - 3: Flow cytometry dot plots in a case of HCL illustrating a clonal B cell population. The neoplastic cells co-express CD103, CD23, CD11c, CD25 and CD123.

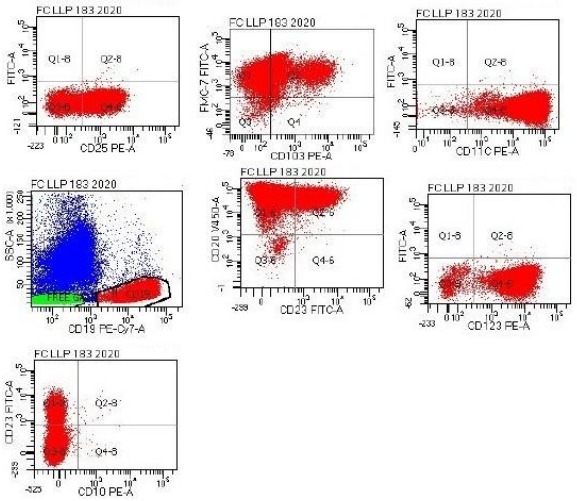

Table – 1: Lab data at diagnosis showed.

Lab Findings	Number of cases
Anemia	5 cases (71%)
Thrombocytopenia	1 case (14.2%)
Leucopenia	2 cases (28.5%)
Monocytosis	3 cases (42.6%)
Pancytopenia	1 case (14.2%)
Leukocytosis	1 case (14.2%)

Table - 2: Physical Examination Findings.

Findings	No. of cases	Percentage
Hepatomegaly	1	14.2
Splenomegaly	2	28.5
Lympadenopathy	1	14.2

Table - 3: Immunophenotypical analysis on Flow Cytometry.

Surface marker	Positive in number of cases	%
CD20	7 cases	100%
CD200	7 cases	100%
CD5	0 cases	--
CD10-	2 cases	28.5%
CD23	3 cases	42.6%
CD11c	7 cases	100%
CD 25	7 cases	100%
CD103	7 cases	100%
CD123	7 cases	100%
CD22	7 cases	100%
FMC	7 cases	100%
Anti-kappa	3 cases	42.6%
Anti – Lambda	4 cases	56.8%

Discussion

Hairy cell leukemiaconstituites approximately 5% of all chronic lymphoproliferative disorders at our institute compared to 2% western literature [1]. The reason is more referral to our center.

Our study showed 7 cases of Hairy cell leukemia in which median age is 52 years where as a French study has 67.8 years and 47 years in a Malfusion JV study [5] and Chatterji T, et al. [6].

Youngest patient in our study was 34 years old whereas other studies like in Komal S Galani, et al. it was 26 years old and 22 years in Bouroncle [7, 8].

Male to female ratio was 6:1comparable with Galani, et al. study, Symptomatic patients in our study showed fever and weakness comparable with Bouroncle and Hoffman study [7, 9].

On clinical examination, splenomegaly was seen in (42.6%) and hepatomegaly in (14.2%). Hepatomegaly is less compared to other studies [10 11]. Golomb and colleagues showed lymphadenopathy in 10% cases which was comparable with our study [11].

Thrombocytopenia was seen in 71% cases in our study which was uncommon in other studies. Pancytopenia was less in our study seen in only one case (14.2%) where as it is 50% in Galani, et al. [7]. Leucocytosis and lymphocytosis was seen rarely in other studies where as it was 14.2% in our study.

Diagnosis of hairy cell leukemia is critical because therapy with purine analogues is associated with high response rate and relapse free survival than in patients and other chronic lymphoproliferative disorder. CD10 positivity in our cases is 28.5% where as it ranges from 5-26% in other studies [12, 13, 14]. CD10 expression is not commonly seen in classical Hairy cell leukemia. We noticed 2 cases with CD10 expression indicated follicular centre of origin.CD23 positivity was seen in 2 cases (28.5%) correlating with other studies [13].

Bone marrow aspirate was aparticulate and hemodiluted in 4 cases. Flow cytometric analysis done on peripheral blood sample or marrow samples. All the marrow biopsies showed lymphocytosis but no classical fried egg appearance seen.Flow cytometry confirmed hairy cell leukemia with help of 4 back bone markers (CD 103, CD123, CD25 and CD11C). BRAF mutation using molecular diagnostic technology by real time polymerase reaction (rt_pcr) was performed in this case which turned out to be positive.

CD123 is used to distinguish between classical Hairy cell leukemia from its variants.CD123 may become negative in cases of Hairy cell leukemia due to covid positivity. The reason is CD123 is an anti-interleukin-3 receptor alpha chain (IL-3RA) antibody which is a part of the cytokine storm associated with covid-19 infection leading to its loss and also due to drugs used in covid like Toclilizumab , methyl prednisolone and IvIgas per Saman Kohla, et al. study which had hairy cell leukemia cases with Covid infection showed CD123 loss, however concluded no association between CD123 and Covid infection and treatment [15]. Our study which had 2 cases of

covid positivity showed CD 123 positivity unlike Kohla study.

Conclusion

Unusual features with leucocytosis, presence of lymphadenopathy and absence of spleen can be present in Hairy cell leukemia. Peripheral blood and marrow aspirate smears should be examined carefully for hairy cell morphology in cases of hemodiluted and aparticulate marrow. Classical morphology of fried egg appearance may not be seen on biopsy morphology. Flow cytometry analysis with CD markers (CD123, CD103, CD25 and CD11C) in correlation with hairy cell morphology along with splenomegaly directs us towards the diagnosis of Hairy cell leukemia.

There was no difference in the patients with covid positivity in the CD markers expression like CD 123 compared to other cases of hairy cell leukemia in other studies which showed CD123 loss due postulated hypothesis of cytokine storm in covid patients with Hairy cell leukemia. However, more research is needed to establish the pathogenesis behind the CD123 loss in covid patients.

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