



ROLE OF CD68+ TUMOUR ASSOCIATED MACROPHAGES IN PROGNOSTICATION OF PATIENTS DIAGNOSED OF HODGKIN'S LYMPHOMA: A PROSPECTIVE COHORT STUDY

Dr. Akshay Sharma*

MD Medicine, Medical officer, Civil hospital Baijnath, kangra (HP).
*Corresponding Author

Dr Anshu Sharma

Medical officer community health centre, Choori Distt chambha (HP).

ABSTRACT

Hodgkin's lymphoma (HL) constitutes around 30% of all lymphomas. IPS is used for the risk stratification of advanced stage HL but it lack applicability to the early stage cases. In this prospective cohort study, we aim to confirm the relationship between CD68+ Tumour Associated macrophages with treatment response (interim as well as end of treatment) of HL treated with the standard chemotherapy and radiotherapy regimens. A total of 50 patients were recruited over a period of 18 months and the patients were followed up in the hematology clinic and interim treatment responses as well as end of treatment response were assessed. Correlation of the CD68+ Tumour Associated Macrophages and the treatment response showed that the Higher Percentages of CD68+ Tumour Associated Macrophages were associated with more advanced stages. Univariate analysis showed that there was no significant correlation of interim treatment response as well as end of treatment response with CD68+ Tumour Associated macrophages levels.

KEYWORDS :

INTRODUCTION

Classical HL represent nearly 95% of all the cases of HL(2). A cure can be achieved in approximately 80% of the patients with current treatment regimen consisting of multi agent chemotherapy and radiotherapy (in selected cases). The remaining 20% attaining remission(4). Early identification of the patients who would fail treatment or relapse in future is currently a challenge. At present the most commonly used tool for prognostication of HL cases is international prognostic score(IPS) (on a scale of 0 -7), with higher scores indicating poorer prognosis). This score is a combination of clinical and basic haematological parameters recorded at the time of diagnosis of a case like age, gender, stage, haemoglobin level, albumin level and lymphocyte counts. Children and adolescents with classical Hodgkin's Lymphoma (HL) have an excellent prognosis. The tumor microenvironment has a critical role in the development and progression of tumors and it also modifies the clinical outcome of patients with hematological malignancies, especially those with follicular lymphoma and cHL.

However, the genomic profile of the HRS cells is still not well characterized yet due to the relative paucity of these cells within the bulk of the tumor. This has also provoked more studies in the area of tumor microenvironment, especially the TAM and their possible diagnostic, prognostic and predictive values. Another important issue related to HL is that until now the clinical and laboratory parameters used for the diagnosis of cHL do not permit accurate identification of patients with less favorable clinical outcomes [17]. However, recent studies provided evidence that an increased number of tumor infiltrating macrophages is significantly associated with shortened survival in HL patients and about 20% will have relapse and/or refractory disease associated with increasing late toxic effects. Accordingly, the authors concluded that, the number of the infiltrating TAM should be considered a powerful prognostic predictor in patients with HL [18]. Based on these data, the aim of the current study was to assess the role of CD68+ tumor associated macrophages (TAM) in the development and progression of cHL. This will be achieved via 1) assessment of the number of CD68 positive macrophages in tissue biopsies, 2) the expression levels of CD68 and CD20 proteins (by IHC) in tumor samples, and 3) the expression level of CD68-mRNA by Rt-PCR. From the current study, we aim to confirm the relationship between CD68+ (M1) TAMs infiltration and clinical outcome in patients of cHL treated with the standard chemotherapy and radiotherapy regimens.

MATERIAL AND METHODS

Study Design

This was a prospective cohort study carried out in the patients diagnosed with Hodgkin's lymphoma who completed treatment at PGIMER, Chandigarh. Patients were enrolled in the study from the Adult Haematology Clinic PGIMER, Chandigarh prospectively from July 2017 to March 2018 and then they were followed up till November 2018.

DURATION OF THE STUDY

July 2017 – December. 2018

Place Of The Study:

1. Department of Internal Medicine Adult Clinical Haematology Unit PGIMER, Chandigarh
2. Department of Histopathology, PGIMER, Chandigarh

Inclusion Criteria

1. Primary diagnosis of Hodgkin's lymphoma confirmed by a tissue biopsy
2. Adequate lymph node tissue in the paraffin block
3. Treatment naïve patients

Exclusion Criteria

1. Patient previously treated with chemo radiotherapy before coming to PGIMER, Chandigarh.
2. Patients who did not complete chemotherapy or end of treatment chemotherapy response assessment were excluded from further analysis.

METHODOLOGY

Procedure

1. Demographic and disease data were recorded in a predesigned performa (annexure 3) and investigations were recorded as per the performa.
2. Collection of the clinical and the laboratory data of the newly enrolled cases was done in the study performa.
3. Biopsy numbers of the lymph node were taken from the Adult Haematology Clinic case record files and the paraffin block were retrieved from the archive of the Department of Histopathology.
4. Cases with adequate tissue in the paraffin block in the archives of the Department of Histopathology were selected and immunohistochemistry staining was performed.
5. ESR levels were done before starting the treatment of the

patients fulfilling the inclusion criteria.

6. Correlation of ESR levels , and treatment response (interim as well as end of treatment response was assessed.

7. The interim treatment response was assessed with the PET CT SCAN after two cycles of the chemotherapy and the response was assessed by the Deauville criteria.

8. The end of treatment response was assessed with the PETSCAN after completion of chemotherapy and the response was assessed by Deauville criteria.

Statistical Analysis

The descriptive statistics were used to study the response rates, Correlation was assessed between ESR levels and the response rate by using Chi square test. Differences were considered as significant if the computed p value was less than theoretical p value i.e. 0.05. Univariate analysis was performed to assess the association between the ESR levels with the treatment response with SPSS, version 22.0.

RESULTS

During the study period, we recruited 60 consecutive, treatment naive patients of Hodgkin's lymphoma. Out of this cohort, there were few exclusions as following:

- A. Three patients died before starting treatment.
- B. Seven patients lost to follow up before completion of therapy.

Hence, their response ~to chemotherapy was not available (due to leaving the treatment before completion). Therefore, in the end, total 50 patients were selected. The patients were followed up in the hematology clinic and interim treatment responses as well as end of treatment response were assessed.

CD68+ Tumour Associated Macrophages:

Majority of the patients had low percentages(<25%) of CD68+TAMs.On subdividing into various subtypes, majority of the patients of HL nodular sclerosis (N=27(71%)) and HL mixed cellularity (N=5(55.5%)) were having low percentages of CD68+ TAMs. Higher percentages (>25%) of CD68+ TAMs were in the advanced stages of disease (stage 3-4).

Table 1 Percentages Of CD 68+ TAMs

PERCENTAGES OF CD68+ TAMs	FREQUENCY (N%)
0 -25%	34(65.4%)
25%-50%	15(28.8%)
50%-75%	3(5.8%)
Total	52

Analysis:

Interim Treatment Response:

Univariate analysis showed that there was also no significant correlation with the percentages of CD68+TAMs with response of interim assessed.

End Of Treatment Response:

Univariate analysis showed that there was also no significant correlation with the percentages of CD68+TAMs with response of end of treatment assessed.

DISCUSSION

In this study, which is the prospective investigation carried out over a period of 18 months based on data available , we observed no association between CD68+Tumour Associated Macrophages concentration and overall risk of Hodgkin's lymphoma . These data are in concordance with recent published studies,reported that intratumoral CD68 macrophage infiltration and B cell markers (CD20) expression can accurately predict DFS and OS rates in the HL patients.

Kamper et al. [25], also reported that High numbers of CD68+ and CD163+ macrophages in cHL associated significantly with worse OS through their correlation with the presence of Epstein– Barr virus (EBV) in the neoplastic cells which in turn, lead to worse outcomes mainly in older individuals [26] . Another study done by Gotti et al. demonstrated that a proportion of tumor-infiltrating macrophages (by IHC) greater than 25% is associated with unfavorable clinical outcome and shorter DFS in 106 early stage cHL patients from Italy. These data are contradictory to that observed by Azambuja et al, who found that there was no significant association between CD86+ /or CD163+ TAM (by IHC) and the relevant clinicopathological features of the patients, OS and DFS rates in 265 well characterized cHL patients from USA. Although present study put forward some evidence which bridge the gap of existing knowledge about role of M1 and M2 tumor related macrophages phenotype in treatment response in patients with HL, there still exist some limitation in present study.

CONCLUSION

In conclusion, higher levels of CD68+Tumour Associated Macrophages were associated with advanced stages. There was no significant correlation between CD68+ Tumour Associated Macrophages percentages with the treatment response interim as well as end of treatment response.

Characteristics Of Patients With Interim Treatment Response

Table No 1. Univariate Analysis Of Interim Treatment Response With Various Prognostic Variables Shows No Significant Correlation (P Value <0.05)

Features	Number of patients in complete remission N (%)	Number of patients not in complete remission N (%)	P value
CD68+TAMs Percentages			
0 -25%	27(69.2%)	6(66.6%)	0.285
25-100%	12(30.7%)	3(33.3%)	

Characteristics Of Patients With End Of Treatment Response

Table No 2. Univariate Analysis Of End Of Treatment Response With Various Prognostic Variables

Features	Number of patients in complete remission N(%)	Number of patients not in complete remission N(%)	P value
ESR levels			
0-25%	32(66.6%)	2(50%)	0.583
25-100%	16(33.3%)	2(50%)	

REFERENCES

1. Rughbjerg K, Maraldo M, Aznar MC, Cutter DJ, Darby SC, Specht L, et al. Long-term hospitalisation rates among 5-year survivors of Hodgkin lymphoma in adolescence or young adulthood: A nationwide cohort study. International journal of cancer. 2017;140(10):2232-45.
2. Ph D, Chan WC, Gascoyne RD. NIH Public Access. 2010;362(10):875–85.
3. The role of CD68+ macrophage in classical Hodgkin lymphoma patients from Egypt Osama Mohamed1 , Ahmed El Bastawisy1 , Nasr Allahlobil , Mona S. Abdellateif2 , Abdel Rahman N. Zekri3 , Sabry Shaarawy2 , Zeinab Korany2 , Marwa Mohanad4 and Abeer A. Bahnassy
4. Prognostic value of tumor-associated macrophages in classic Hodgkin's lymphoma: systematic review and meta-analysis Tao Jiang1,2, Xiaomin Cheng1, Yongqian Jial