ORIGINAL RESEARCH PAPER

Pathology

DETECTION OF BONE MARROW MICROMETASTASIS IN CASES OF GASTRIC MALIGNANCIES

KEY WORDS: bone marrow , disseminated tumor cells , gastric carcinoma .

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ABSTRACT

Gastrointestinal malignancies are among common cancers in humans. Surgical resection remains treatment of choice but despite surgery 45% ultimately die of distant metastasis. Detection of Disseminated Tumor Cells may provide a promising approach to improve prognosis and for changing treatment approach in patients. Our aim is to study the incidence of bone marrow micrometastasis in patients with gastric cancers and to correlate the presence of micrometastasis with other prognostic factors .We performed study in 50 patients with gastric malignancies. 9 patients (18%) showed positivity for disseminated cells in bone marrow . Depth of tumor invasion, stage and histological grade of tumor correlated statistically with presence of disseminated cells in marrow .

INTRODUCTION:

Adenocarcinoma of stomach is the fourth most common cancer and second most common cause for death due to cancer throughout the world[1] . Recurrence of tumor even after complete removal of primary is an important problem in management of patients with carcinoma stomach[1]. Gastric carcinoma usually has a bad prognosis with a 5 year survival rate of 10% - 55% even after curative resection[2].

Adverse prognostic factors are old age (>70 years) , Tumor location (proximal worse than distal), presence of venous /lymphatic invasion, CEA >10µg/ml, CA-19-9 > 37µg/ml. Intestinal type carcinomas have better prognosis than diffuse type as they present early[2]. Pathological staging (TNM) is the most powerful predictor of outcome, based on depth of invasion , presence or absence of nodal metastasis and distal metastasis.

Micrometastasis is defined as metastases seen only at microscopic level, i.e. not overtly evident (<0.2cm)[3,4] . The bone marrow is generally viewed as the optimal site to search for micrometastases as it is a rich cellular site with increased blood supply and because cytokeratin positive epithelial cells are easily identifiable in mesenchymal tissues[5,6.] . However, it seems likely that other organs such as liver and lung also harbour dormant micro deposits of the residual disease. There have been several reports describing bone marrow micrometastasis in subjects with carcinoma of lung , prostate, mammary carcinoma , stomach, pancreas, colon and squamous cell carcinomas of the head and neck region [3,7].

Numerous prospective clinical studies have confirmed the prognostic significance of immuno cyto chemical detection of occult cancer cells in bone marrow, many of these studies also have stated the detection of these disseminated cancer cells as an independent risk factor [8]. The disseminated cells are detected even in marrow of those epithelial tumors that normally do not cause skeletal metastasis . Presence of these cells in marrow act as a window to denote that the cells could have spread to other sites also including the site of final metastasis[8,9] .

Identification of the malignant epithelial cells in bone marrow , peripheral blood or lymph nodes which are mesenchymal tissues is aided by cytokeratin which is now the commonly used epithelial marker[10,11,12] .

Gastroesophageal cancer, shows a wide range of incidence of disseminated tumor cells ranging from 29%–67%. The presence of disseminated cells in bone marrow of gastric carcinoma patients was found to be related to stage of the tumor and it predicted both Disease Free Survival and Overall Survival .

AIMS AND OBJECTIVES:

To study the incidence of bone marrow micrometastasis in patients with proven gastric malignancies.

To study the prognostic significance of bone marrow micrometastasis in relation to other prognostic factors of gastric malignancies

MATERIALS & METHODS:

This study was proposed and conducted in the Government Tirunelveli Medical College Hospital, Tirunelveli . Patients who presented to the outpatient department of the Departments of Surgery and Oncology at during the period of September 2011 to July 2013 with biopsy proven diagnosis of malignancy in stomach were evaluated. Of these, 50 patients were selected for our study based on a set of inclusion and exclusion criteria.

INCLUSION CRITERIA:

- Patients with biopsy proven Gastric malignancy
- Patients without any radiological lesion
- Patients with/without lymphatic spread.

EXCLUSION CRITERIA:

- Patient with obvious secondary metastasis
- Patient with radiologically confirmed metastasis
- Patient undergoing chemotherapy/radiotherapy
- History of present/previous other tumors.

Patients were followed and surgical recection specimens received were evaluated .Careful gross examination for size of tumor , depth of invasion and extensive search for lymph nodes made . Sections were processed and studied to note the microscopic type of tumor , grade , depth of invasion and number of nodes positive for metastatic carcinomatous deposits under light microscopy.

Patients were followed post operatively and before administration of first cycle of chemotherapy bone marrow aspirations were taken . Written consent was obtained from the patient or his/her nearest relative after proper counselling. Bone Marrow aspirate was obtained from the 50 patients selected for the study. Bone Marrow aspirate was obtained by direct puncture of bilateral posterior iliac crests under local anaesthesia with strict aseptic precautions. Marrow Smears were prepared and fixed in methanol, of which 3 smears were stained with Leishman stain .

The cases were evaluated for disseminated tumour cells.The Bone Marrow smears of positive patients were further evaluated using Pan Cytokeratin Stain.

The results and observations were tabulated, analysed and statistically evaluated for their significance.

OBSERVATION AND RESULTS :

Fifty cases of gastric carcinoma were included in study . Bone marrow aspirations were done and processed and reviewed for presence of bone marrow disseminated tumor cells (DTC). Nine patients had bone marrow micro metastasis .

TABLE 1:INCIDENCE OF MICROMETASTASIS.

Total no of cases	50
No of cases positive for DTC	9(18%)
No of cases negative for DTC	42(82%)

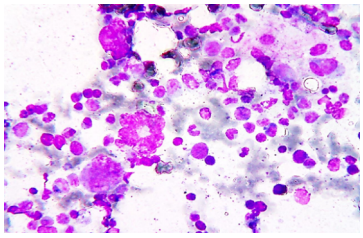

FIGURE 1 : photomicrograph showing malignant epithelial cells forming acinar clusters(Leishman x40)

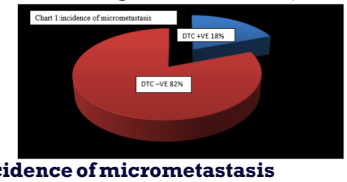

Chart 1:incidence of micrometastasis

TABLE 2:TUMOR DEPTH AND DTC.

Depth of the tumor (T)	Total No. of cases	No. of DTC + VE cases	No. of DTC - VE cases
T1	-	-	-
T2	24	-	24(100%)
T3	26	9(34.6%)	17(65.4%)
T4	-	-	-

Of 50 cases studied there were no cases of early gastric carcinoma. Most of them were T2 and T3 lesions extending to muscularis propria and serosa respectively. Of these no case of T2 lesion were positive and 9 cases of T3 lesions were positive for disseminated tumor cells in marrow .

TABLE 3:NODAL STATUS AND DTC.

Nodal Status (N)	Total No. of cases	No. of DTC + VE cases	No. of DTC - VE cases
N0	8	-	8(100%)
N1	34	4(11.76%)	30(88.23%)
N2	6	4(66.66%)	2(33.33%)
N3	2	1(50%)	1(50%)

Majority of patients , nearly 34 were N1 of whom 4 were positive for DTC. Among six patients in N2, 4 were positive and one among two with N3 was positive for DTC . 8 patients were in N0 stage and none of them were DTC positive .

TABLE 4: GRADE OF TUMOR AND DTC:

Grade	Total No. of cases	No. of DTC + VE cases	No. of DTC - VE cases
Well differentiated	22	-	22(100%)
Moderately differentiated	10	2(20%)	8(80%)
Poorly differentiated	18	7(38.88%)	11(61.11%)

Of 50 patients studied 22 were well differentiated , 10 cases were moderately differentiated and 18 patients had poorly differentiated carcinomas. None of well differentiated tumors were DTC positive.20% (2)of moderately differentiated tumors and 38.88% (7)of poorly differentiated tumors were DTC positive .

TABLE 5: STAGE OF TUMOR AND DTC:

TNM STAGE	Total No. of cases	No. of DTC + VE cases	No. of DTC - VE cases
I	10	-	10(100%)
II	12	-	12(100%)
III	24	7(29.16%)	17(70.83%)
IV	4	2(50%)	2(50%)

Of 50 cases studied 10 were in TNM stage 1, 12 in stage 2. 24 cases were in stage 3 and only 4 cases in stage 4. No cases from stage 1 and stage 2 were positive.7 out of 24 cases in stage 3 and 2 out of 4 cases in stage 4 were positive for disseminated tumor cells .

DISCUSSION :

The results of our study were compared with other similar studies .

TABLE 6: COMPARISION OF DETECTION RATES :

STUDY	DETECTION RATE
Our study	18%
Panabiereset al (2008)[10]	35-60%
Pantel et al (1999)[8]	31.3%
Sullivan et al(1995)[13]	27%

Thus our study is comparable with most other studies discussed . But proportion of positive bone marrow DTC is low in our study compared to others .Higher rate of detection in other studies compared to our study may be attributed to difference in methods used to detect micrometastasis . This may also be attributed to factors like lack of enrichment techniques, short period and small study population .This study yet confirms that the independent prognostic value of detection of DTC in Bone Marrow in gastric cancer is significant and has to be studied widely in the Indian context with specific references to the regional, racial and socio-economic factors that may play a significant role.

CONCLUSION

Our study has highlighted the following important observations indicating the importance of DTC in prognosis of gastric malignancies .

A. Significant proportion of patients with gastric malignancies have disseminated tumor cells in bone marrow which is evidence of minimal residual disease .
B. Presence of disseminated tumor cells showed increased incidence with increase in depth of invasion of tumor,increase in nodal status and stage of tumor .
C. Poorly differentiated tumors had higher incidence of disseminated cells than differentiated tumors.
D. Our study also concludes that disseminated tumour cells in Bone Marrow could have an independent prognostic value and has to be studied widely in the Indian context with specific reference to the regional, racial and socioeconomic factors.

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