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Original Research Article

A study to assess the therapeutic role of GM-CSF (EMGRAST -M) on augmentation of total leucocyte count and total platelet count in cancer patients after chemotherapy

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ABSTRACT

Background: The aim of the present study was to assess the therapeutic role of GM-CSF (EMGRAST -M) on augmentation of total leucocyte count and total platelet count in cancer patients after chemotherapy.

Methods: The total leucocyte count (TLC) and total platelet count (TPC) of thirty patients on chemotherapy were obtained before and after the administration of GM-CSF. The results were analysed retrospectively for the effect of GM-CSF on these parameters. Statistical analysis was done, and graphs were made by Libre office calc and Student's T Test was used for comparison of data.

Results: The study showed that EMGRAST-M had an impressive effect on both the platelet count and the leucocyte count.

Conclusions: GM-CSF has a great therapeutic role in the enhancement of platelet count and leucocyte count in patients of cancer chemotherapy.

Keywords: Chemotherapy, GM-CSF, Total leucocyte count, Total platelet count

INTRODUCTION

The colony stimulating factors (CSFs) are cytokines that stimulate the formation of maturing colonies of leucocytes observable in tissue culture.

They not only stimulate particular committed progenitor cells to proliferate but also cause irreversible differentiation. The responding precursor cells have membrane receptors for specific CSFs and may express receptor for more than one factor, thus permitting collaborative interactions between factors.¹

Granulocyte stimulating factor (G-CSF) was among the first cytokines to be identified and to enter clinical trials, way back in 1960s. But Human G-CSF was purified from the conditioned medium of the bladder carcinoma cell line 5637 by Carl Welte in 1985. In 1986, G-CSF gene was cloned in Japan, permitting the large scale production of this cytokine and its subsequent clinical application.²

Granulocyte-macrophage colony-stimulating factor (GM-CSF) also known as CSF-2 is a monomeric glycoprotein secreted by macrophages, T-Cell, mast-cell, natural killer cells, endothelial cells and fibroblasts, Emgrast-M (sargramostim) is the pharmaceutical analogue of naturally occurring GM-CSF, yeast derived rHu GM-CSF produced using Saccharomyces cervisiae and is classified as a multilineage CSF. Due to this ability, this drug is used in patients after autologous bone marrow transplantation (AuBMT), peripheral blood progenitor cell transplantation (PBPC), induction therapy for acute myelogenous leukemia (AML). These uses are well established and have been recently reviewed.³ The lifesaving chemotherapy and radiation can also result in male infertility. The cytokine G-CSF promotes spermatogenic regeneration from surviving spermatogonia after high dose alkylating chemotherapy in a manner that involves enhanced proliferation of undifferentiated spermatogonia. 4 G-CSF is also used to treat isolated congenital, cyclic and idiopathic neutropenia and some cases of myelodysplastc syndromes and aplastic anemia, reducing symptoms like mouth ulcers, febrile events and infections.2

Emgrast-M functions as a cytokine- it is a white blood cell growth factor. It stimulates stem cell to produce granulocytes (neutrophils, eosinophils, basophils) and monocytes. Monocytes exit the circulation and, in the tissue, mature into macrophages and dendritic cells. Thus, it is a part of immune cascade which is crucial for fighting infections. It also causes delayed type of hypersensitivity and may have a potential role as an anti-viral vaccine adjuvant, anti-tumour agent, in mucositis, stomatitis and diarohhea. ^{5,6}

The chemotherapeutic agents used in the treatment of cancer are well known for causing bone marrow suppression. The fall in haemoglobin count does not make so much impact on the general health and further treatment protocols than the total lecucoyte count and the platelet count. Patient may go into febrile neutropenia which can be life-threatening.

The capacity to increase bone marrow cellularity and peripheral neutrophil count by the use of G-CSF has been shown in various clinical studies. Interesting to note here is that the haematopoeitic system of GM-CSF deficient mice appears to be normal, with some significant changes in T cell response only.8Stimulation of polymorphonuclear neutrophils may increase the host defence against infection. At the same time, it may also enhance tumor control. 9

With this background, the present assessment was done to assess the role of GM-CSF on total leucocyte count and platelet count in cancer patients on chemotherapy.

METHODS

This was an observational retrospective analysis conducted among the patients receiving chemotherapy in Meherbai Tata Memorial hospital, Jamshedpur in Jharkhand during November 2009 to August 2016. Those patients were selected who were suffering from various types of cancers and were on different chemotherapy protocols. This study was conducted under the aegis of Declaration of Helsinki and approval of institution ethics committee was taken before initiating the study.

Inclusion criteria

- The sample included patients from either sex
- They were selected randomly to minimize selection bias.

Exclusion criteria

Those patients who were on other drugs which can cause an increase in these parameters; for example, steroids, were excluded from the study.

Their complete blood count after the chemotherapy was noted down. The 30 patients who showed a marked decrease of total leucocyte count and total platelet count were chosen for the study. They were administered injection Emgrast-M 500 micrograms subcutaneously in three dosages on three consecutive days. This is sargramostim manufactured by Emcure Pharmaceuticals Ltd. and its MRP is INR 3077 per vial. The complete haemogram on the day subsequent to the administration of all the three dosages of GM-CSF was noted down.

Statistical analysis

Statistical analysis was done after entering the data in Libre office calc. The results were expressed with the help of paired- T test. Bar graphs to show the changes in the two parameters was separately plotted. The level of significance for both the parameters was <0.05.

RESULTS

The patients suffering from different types of cancers who were treated with GM-CSF after chemotherapy responded very well in most of the cases. Total leukocyte count was definitely augmented. So also, total platelet count was markedly increased. In this study, the male to female ratio was 13:17, i.e. it was inclined more towards females.

Figure 1 shows the data of total leucocyte count of each patient pre and post intervention i.e. it denotes the absolute cell count both before and after the study. From the bar

graph, the marked change in the TLC in each case can be duly appreciated and analysed.

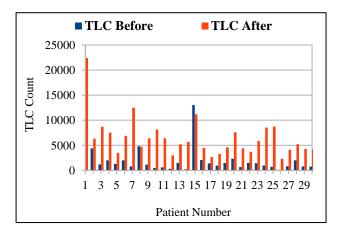


Figure 1: TLC comparison pre and post intervention.

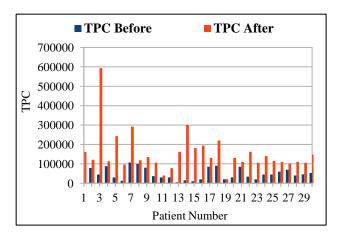


Figure 2: Comparison of TPC pre and post intervention.

Figure 2 shows the data of total platelet count of each patient pre and post intervention. Here again, by analysing the graph, the shift in TPC that occurs after administration of Emgrast-M can be assessed.

To compare the mean extent of change in total leucocyte count before and after the use of GM-CSF, Paired T test was used. The mean, variance and P-values have been calculated by the use of Libre Office Calc. These observations are being expressed in the Table 1.

Similarly, the mean extent of change in total platelet count before and after the study was recorded. By the use of Paired T test, again the mean, variance and P-values have been calculated. These observations are being expressed in the Table 2. The power of the study has been calculated for both the parameters. The value in both the cases is 0.05 and has been shown in the respective Tables.

P value and statistical significance for total leucocyte count is as follows. The two-tailed P value is less than 0.0001 By conventional criteria, this difference is considered to be

extremely statistically significant. The mean of TLC Before minus TLC after equals -4674.17. The 95% confidence interval of this difference lies in between -6246.76 to -3101.58.

Table 1: T-test results for total leucocyte count.

Observations	TLC results	
Alpha	0.05	
Hypothesized mean difference	0	
	TLC before	TLC after
Mean	1744.67	6418.83
Variance	5660942.99	15071744.28
Observations	30	30
Pearson correlation	0.1621842924	
Observed mean difference	-4674.1666666667	
Variance of the differences	17736527.7	
Df	29.0000000000	
t Stat	-6.0789772798	
P (T<=t) one-tail	0.0000006414	
t Critical one-tail	1.6991270265	
P (T<=t) two-tail	0.0000012828	
t Critical two-tail	2.0452296421	

Table 2: T-test results for total platelet count.

Observations	TPC results	
Alpha	0.05	
Hypothesized mean difference	0	
	TPC before	TPC after
Mean	47153.33	154856.67
Variance	918332919.54	10747237022.99
Observations	30	30
Pearson correlation	0.03	
Observed mean difference	-107703.33	
Variance of the differences	11454441712.64	
Df	29	
t Stat	-5.5119187694	
P (T<=t) one-tail	0.0000030607	
t Critical one-tail	1.6991270265	
P (T<=t) two-tail	0.0000061214	
t Critical two-tail	2.0452296421	

P value and statistical significance for total platelet count is as follows. The two-tailed P value is less than 0.0001 By conventional criteria, this difference is considered to be extremely statistically significant. The mean of TPC Before minus TPC after equals -107703.33 The 95% confidence interval of this difference lies in between -147667.30 to -67739.37.

Collectively, these results indicate that G-CSF administration exogenously plays a critical role in the management of chemotherapy induced complications pertaining to the haematological systems and is greatly helpful for the patient to overcome them.

DISCUSSION

Both IL3 and GM-CSF effect megakaryocyte colonies. Some in-vitro studies have shown additive effects. ^{10,11} Here dose dependent significant increase in platelet numbers in vivo in non-human primates injected first with IL3 and then with GM-CSF was seen. However, when each substance was injected alone, no significant change in platelet number was observed. This seems to confirm the additive effects of GM-CSF and IL3 in vitro and in vivo.

A phase I/II trial of sequentially administered IL3 and GM-CSF was reported in 9 patients with myelodysplasia; 6 were dependent on red cell transfusion and 2 were dependent on platelet transfusions.³ Patients were given IL3 followed by GM-CSF on a 28 days cycle. They received IL3(1mcg/Kg/d) on days 1 to 7, followed by GM-CSF (3 mcg/Kg/day) on days 8 to 21. Here, the effects on platelets were minimal.

The number of studies which relate only to the effects of GM-CSF on haematological parameters are few and not very conclusive.

According to the results in this study, GM-CSF appears to be very suitable for the augmentation of total leucocyte count and total platelet count in the cancer chemotherapy patients.

This study can further be cross validated on different data sets and by increasing the sample size. It can be further improved upon by doing separate tests on separate cancer types. Demographic factors like male and female variations and different age groups can also take into account. Unfortunately, some toxic side effects secondary to GM-CSF have been reported. Malaise, nausea, night sweats and bone pain are the common ones. Serious events like splenic rupture, interstitial pneumonitis, pulmonary infiltrates, lung fibrosis and respiratory distress syndromes have been described. Infiltration of neutrophils in target tissues is characteristic of many inflammatory conditions, with neutrophils being the major leukocyte found in rheumatoid and collagen induced arthritis. These should also be taken into consideration.

The small number of clinical studies, the heterogeneity of the study groups and the lack of selection criteria are some of the reasons for the lack of clear views in medical literature. There are still many questions to be answered. What is the best route of administration? What group of patients will be the most benefitted by the treatment? What should be the most efficacious dose and the level of blood cells in which the treatment should be initiated. Can some more cost-effective products be designed for use in under-

developed countries. Well-designed clinical studies should be conducted to answer these questions.

G-CSF administration appears to be associated with an increase in regulatory T cells and dendritic cells and appears to influence endometrial expression of genes crucial for the implantation process, including endometrial vascular remodelling, local immune modulation and cellular adhesion pathways. Thus, treatment with this agent is a novel proposal for immune therapy in patients with recurrent miscarriage and implantation failure following cycles of IVF.¹⁴

Topically applied plant-derived rhGM-CSF has been found to increase the wound-healing rate in vivo. ¹⁵ This possibility of developing rhGM-CSF as a treatment option for corneal epithelial wounds should be explored. The role of the receptor CXCR2 signalling in mediating neutrophil release from the bone marrow along with GM-CSF and independently also can be an interesting field of study. ¹⁶

Thus, it can be used therapeutically in cancer chemotherapy patients to save them from the disasters of bone marrow suppressions due to the chemotherapeutic agents. Febrile neutropenia which can be lead to fatality can be successfully avoided by using GM-CSF at appropriate time in appropriate dosage when the patient is being carefully monitored.

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Institutional Ethics Committee

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