Granulocyte Macrophage Colony Stimulating Factor Level in Serum of Patient with Hodgkin Lymphoma Pre and Post-Chemotherapy

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1. INTRODUCTION

Hodgkin lymphoma (HL) is an uncommon B-cell lymphoid malignancy (Ansell, 2014). Hodgkin and Reed-Sternberg cells are present within a cellular infiltrate of non-malignant inflammatory cells making the majority of the tumor tissue. An accurate assessment of the disease stage is critical for the selection of the appropriate therapy (Swerdlov et al., 2008). The factors that play role in the clinical and pathologic features of HL are cytokines (Skinnider and Mak, 2002).

Cytokines play a vital role in the regulation of key pathways of immunity, the balance between both arms of immunity, cell-mediated (Th1) and humeral (Th2) responsiveness (Spilianakis et al., 2005). Since immune dysfunction is thought to be the underlying basis of lymphomagenesis, an
imbalance in the regulation and expression of Th1 and Th2 cytokines could play a central role in the etiology of lymphoma (Chiu and Weisenburger, 2003; Mori et al., 2001).

Granulocyte-Monocyte Colony stimulating factor (GM-CSF) stimulates stem cells to produce monocytes and granulocytes. Monocytes migrate into tissue for mature into dendritic cells and macrophages (Cruz et al., 2014). The GM-CS function in the supportive care of cancer patients has been evaluated with a promising outcome (Arellano and Lonial, 2008). Indeed it has been used to speed up healing of the granulocyte and monocyte counts after chemotherapy (Moore, 1991). The immunostimulatory properties of GM-CSF may be beneficial in reconstituting the compromised immune system as indicated by numerous research (Hartung et al., 2000). To the regimens for the mobilization of hematopoietic progenitor cells, GM-CSF has been added. Indeed, it has another role in regulating immune responses by its effect on antigen presenting cells development and maturation. GM-CSF is skewing the immune system toward Th1-type responses leading to initiating cytotoxic immune responses. The utilization of GM-CSF in combination with cytotoxic or other targeted therapies are required for the successful use of myeloid acting cytokines to increase anti-tumor responses (Arellano and Lonial, 2008). It is well-known that GM-CSF has a vital role in the pathogenesis of lymphoma patients (Tekei and Gulbas, 2014). It used commonly to support granulocytes or antigen presenting cells production (Mehta et al., 2015).

In this study, we aimed to determine a GM-CSF serum cytokine levels, their prognostic significance in patients with HL comparison with NHL and healthy control groups. Indeed evaluate their level before and after different chemotherapy doses in lymphoma patients.

2. MATERIALS AND METHODS

Study groups were classified into 40 HL patients and 40 NHL as patient control and 40 healthy control (HC), they were apparently healthy and chosen to match the age and sex of the patient group during May to November 2015. Five ml of blood samples were taken from lymphoma patients who were admitted to Baghdad Teaching Hospital and hospitals staffs. Some patients had not received any chemotherapy doses; however, other lymphoma patients received variance doses of chemotherapy. The study protocol includes estimation of GM-CSF serum level (pg/mL) by using immunological assay, Enzyme-linked immunosorbent assay (ELISA) (Marseille Cedex 9 /France) as quantitative detection.

Analysis of data was performed by using Statistical Package for Social Science (SPSS) Version 18. The study results expressed as mean ± S.E. Statistical differences were determined by t-test for two comparisons and ANOVA for multiple comparisons. The P value less than 0.05 were considered statistically significant. All procedures were approved by an ethics committee of the College of Medicine/Hawler Medical University and the consent of the participants was obtained.

3. RESULTS AND DISCISION:

Lymphoma patients in this case-control study were divided into two groups 40 had HL (15 female and 25 male) and 40 Non-HL (15 female and 25 male) as a patient control. The mean age was 60.6 ± 13.9 years for HL, and 57.5 ± 12.4 years for Non-HL patients. The control group included 40 healthy individuals (19 female and 21 male) with mean age 48.6 ± 5.3 years. Regarding gender distribution, GM-CSF serum levels were not significantly increased in female than male (5.02 versus 4.71) in HL and (5.28 versus 4.80) in NHL patients (Table 1). The no significantly increased of GM-CSF serum level in female compared with a male in the present study may support the hypothesis that the female immune response more than male (Kim et al., 2008).

Non-significant differences in mean serum level of GM-CSF were observed
between HL, NHL and HC groups (P=0.08) when analyzed by ANOVA. Same result was revealed when compared between HL and NHL (4.80±0.18 versus 4.99±0.22)(P=0.99), inversely significant decreased result was revealed when compared between HL patients(4.80±0.18) and HC (4.10±0.15) (P=0.04). Indeed, the mean serum level of GM-CSF significantly increased in NHL patients(4.99±0.22) compared with HC group (4.10±0.15) (P=0.03) when analyzed by Student t-test (Table 2).

It is acknowledged that cytokines have an important role in the pathogenesis of lymphoma, are potential markers in growth of tumor cells, and when compared with healthy subjects serum cytokine levels are higher in lymphoma patients(Aydin et al., 2002; Fabre-Guillevin et al., 2006) a fact that was also established in our study. This suggests that the mentioned cytokine may be a good marker for monitoring the pathogenses in lymphoma patients.

Decreased in GM-CSF serum levels were reported in HL patients before chemotherapy, zero doses(6.09±1.15)and after receiving 1-6 (4.51±0.17), 7-12 (4.683±0.16)and 13-30(4.682±0.44 ) doses of chemotherapy respectively(P< 0.05). An inverse result was conducted concerning NHL patients (P>0.05) when compare before chemotherapy zero doses(4.47±0.19) and 1-6 (4.90±0.18), 7-12 (4.90±0.18) and 13-30(6.26±0) doses respectively(Table 3).

The results of this study showed that in HL patients after chemotherapy, the serum level of GM-CSF has significant decrease compared to before chemotherapy. Most cytokines serum levels decreased after therapy in common HL patients. However, elevated cytokine levels persisted in patients with complete remission(Skinnider and Mak, 2002). In addition, the study of Hematol, (2014)was reported that in HL patients, some serum cytokines levels decreased after chemotherapy.

The decrease of serum level of the GM-CSF cytokine after chemotherapy is possibly linked with the cytotoxic result of chemotherapy on the blood cells or, probably due to the complex outcome of other cytokines on the mentioned cytokine production(Sepetehrizeh et al., 2014). NHL patients GM-CSF serum level shows a different pattern of serum level, it increased non-significantly after chemotherapy, but there a significant increase in their level after higher doses of chemotherapy. The increase of GM-CSF reached to higher than the levels before chemotherapy, which could be due to the effect of mentioned cytokine in a recovery of the immune system, indeed the stimulation and release of different cytokines(Sepetehrizeh et al., 2014).

Regarding HL patients, their serum level of GM-CSF was decreased before chemotherapy, zero doses and after receiving 1-6, 7-12, and 13-30 doses of chemotherapy respectively. However, in NHL patients an inverse result was conducted when compare before chemotherapy zero dose and 1-6 , 7-12, and 13-30 doses respectively.

Increased pretreatment serum cytokines like GM-CSF are related with elevated disease relapse and inferior survival in lymphoma patients(Marri, 2013). Many researchers also revealed decreased in mean serum level of GM-CSF in patients with HL as opposed to healthy individuals(Teke and Gulbas, 2014; Gorschlüter, 1995), they reported a high level of GM-CSF cytokine in healthy controls in comparison to lymphoma patients. Indeed they found that GM-CSF level rarely detectable in lymphoma patients(Skinnider and Mak, 2002), however, Angela et al., reported the majority of untreated HL patients with elevated IL-6 level also had increased GM-CSF levels and vice versa (Angela et al., 1992). In contrast, increased levels of G-CSF were found in many patients with HL as opposed to healthy individuals. The decreased serum level of mentioned cytokine in HL patients may be due to the suppression of the immune system in those patients and accumulation of abnormal cells blood (Sepetehrizeh et al., 2014) or it could be associated with abnormal Th1 to Th2 subpopulation shifting (Lan et al., 2006).
Table 1: Comparison of GM-CSF mean serum level in lymphoma patients according to the gender

| Study groups             | Female Mean ±SE   | Male Mean ±SE | P value (t-test) |
|--------------------------|-------------------|---------------|-----------------|
| Hodgkin lymphoma         | 5.02±0.56         | 4.71±0.15     | 0.46            |
| Non-Hodgkin lymphoma     | 5.28±0.35         | 4.80±0.28     | 0.30            |

P>0.05: Non significant

P<0.05: Significant , P>0.05: Non significant

Table 2: Comparison of mean serum GM-CSF level in HL, NHL patients and healthy control

| Study groups             | GM-CSF Mean ±SE | P value (F-test) |
|--------------------------|------------------|-----------------|
| Hodgkin lymphoma         | 4.80±0.18        |                 |
| Non-Hodgkin lymphoma     | 4.99±0.22        | P>0.05          |
| Healthy control          | 4.10±0.15        |                 |

HL versus HC 0.04 P<0.05
NHL versus HC 0.03 P< 0.05
HL versus NHL 0.99 P>0.05

HC: Healthy control, HL: Hodgkin lymphoma, NHL: Non Hodgkin lymphoma
Table 3: Comparison of mean serum GM-CSF concentrations in lymphoma patients according to number of chemical doses

| Lymphoma patients | No. of patients | No. of chemotherapy doses | GM-CSF Mean ±SE | P value (t-test) |
|-------------------|-----------------|---------------------------|-----------------|----------------|
| Hodgkin lymphoma  |                 |                           |                 |                |
| 1                 | 10              | 0                         | 6.09±1.15       |                 |
| 2                 | 11              | 1-6                       | 4.51±0.17       | 1 Vs 2=0.01    |
| 3                 | 10              | 7-12                      | 4.68±0.16       | 1 Vs 3=0.02    |
| 4                 | 9               | 13-30                     | 4.67±0.44       | 1 Vs 4=0.03    |
|                   |                 |                           |                 | 2 Vs 3=0.69    |
|                   |                 |                           |                 | 2 Vs 4=0.75    |
|                   |                 |                           |                 | 3 Vs 4=0.99    |
| Non-Hodgkin lymphoma |             |                           |                 |                |
| 1                 | 12              | 0                         | 4.47±0.19       | 1 Vs 2=0.36    |
| 2                 | 10              | 1-6                       | 4.90±0.18       | 1 Vs 3=0.23    |
| 3                 | 9               | 7-12                      | 5.23±0.88       | 1 Vs 4=0.04    |
| 4                 | 9               | 13-30                     | 6.26±0          | 2 Vs 3=0.52    |
|                   |                 |                           |                 | 2 Vs 4=0.12    |
|                   |                 |                           |                 | 3 Vs 4=0.28    |

4. CONCLUSION:
The present result revealed that GM-CSF considers as lymphoma promising monitoring biomarkers, and has an association with chemotherapy doses in HL patients.

ACKNOWLEDGMENT:
I wish to thank all the Doctors and staff members of Baghdad teaching hospital for their kind and valuable assistance.

Conflict of Interest: There is no conflict of interest.

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