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# HUMAN MACROPHAGE COLONY - STIMULATING FACTOR (M-CSF) LEVELS IN PATIENTS WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP)

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## ABSTRACT

We measured serum M-CSF concentrations in 25 patients with idiopathic thrombocytopenic purpura (ITP) to determine if they were elevated in this condition, and to investigate the effect of corticosteroid therapy on the level of this growth factor in 8 ITP patients with profound thrombocytopenia. M-CSF levels were measured by enzyme immunosorbent assay (ELISA). The mean M-CSF was significantly increased ( $P < 0.05$ ) in patients with ITP in comparison to control group (mean + SD were  $929 \pm 127$  u/ml and  $364 \pm 69$  u/ml respectively).

There was a significant negative correlation between M-CSF and plate-

let count ( $P < 0.05$ ). The mean level of M-CSF was significantly decreased three weeks after corticosteroid therapy in comparison to the high pretreatment level ( $P < 0.05$ ). Thus we concluded that the elevated level of M-CSF in children with ITP may reflect activation of reticuloendothelial system which could result in positive feedback to increase the destruction of platelets. Corticosteroid therapy could induce thrombocytosis by decrease of M-CSF level in patients with ITP.

## INTRODUCTION & AIM OF THE WORK

Idiopathic thrombocytopenic disorder is characterized by destructive thrombocytopenia. In this disorder

antibody coated platelets are removed from the circulation in the mononuclear phagocyte system via macrophage FC receptors. 1,2,3 However, the severity of thrombocytopenia may relate to several interacting factors. These include the level and subclass of antibody, the effect of antibody on platelet production, and the status of monocyte macrophage phagocytic system. 4,5,6

M-CSF is a hematopoietic growth factor that has been characterized by its ability to stimulate proliferation, differentiation, and maturation of monocytes. It also enhances several monocytic effector functions, two of which include antibody dependent cellular cytotoxicity and the expression of low affinity IgG receptors. Enhancement of these functions could affect the status of the macrophage in patients with ITP. 7,8

M-CSF is produced by fibroblasts and bone marrow stromal cell culture. These cells are probably responsible for the levels of M-CSF detected in the serum of normal animals and humans. 9 The association between high serum M-CSF levels and thrombocytopenia has been demonstrated in adults. 10,11,12

Exogenous administration of M-CSF in preclinical and clinical trials is associated with thrombocytopenia. 8,13

The present study was undertaken to measure serum levels of M-CSF in 25 children with ITP; aiming to correlate M-CSF with platelet number; and find the effect of corticosteroid therapy on M-CSF level in patients under corticosteroid therapy to induce thrombocytosis.

## SUBJECTS

This study was performed prospectively in pediatric department, Benha University Hospital including 25 children with ITP (11 males and 14 females), aged 1- 6 years, presenting with sudden onset of generalized petechiae and purpura and with history of a preceding viral infection 1-4 weeks before onset of purpura. The physical examination is normal other than the finding of petechiae and purpura, children with abnormal findings such as hepatosplenomegaly or lymphadenopathy that suggest other diagnosis were excluded. Twenty five aged matched healthy children with normal hematological indices were the control group.

## METHODS

- 1- Complete blood cell count including Platelet count.
- 2- Eight patients with platelet count below 30000 / mm<sup>3</sup> where subjected to corticosteroid therapy in dose 1-2mg kg/24hr of predinsone up to 2-3 weeks or until the rise in platelet count above 100000/mm<sup>3</sup> has been achieved.
- 3- Bone marrow examination was done for patients with abnormal white blood cell count or unexplained anemiã, where it revealed normal granutocytic and erythrocytic series with characteristically normal or increased number of immature megakaryocytes.
- 4- Coombs test was done for patients with unexplained anemia to rule out Evan syndrome (autoimmune hemolytic anemia and thrombocytopenia)
- 5- M-CSF measurements: serum was collected after centrifugation of blood sample and stored at-20 oC until the assay was performed. Serum M-CSF levels were measured with an enzyme linked immunosorbent assay (Quantikine ELISA Kit, R&D system, minneapolis, MN,USA). A monoclonal antibody specific for M-CSF had been precoated into a microtiter

plate. Standards and samples were pipetted into the wells and any M-CSF present was bound by the immobilized antibody. After washing away any unbound substance,an enzyme linked polyclonal antibody specific for M-CSF was added to the wells. After a wash to remove any unbound antibody enzyme reagent,a substrate solution was added to the wells and color was developed in proportion to the amount of M-CSF bound in the initial step. The color development was stopped and the intensity of the color was measured quantitavely using ELISA at wave length 450 nm.14 ,15

## RESULTS

Table (1) showed the mean SD of M-CSF in ITP patients and control group. The mean M-CSF was significantly increased in ITP patients in comparison to the control group (P<0.05).

Table (2) expressed the mean  $\pm$  SD of M-CSF level in patients they receiving corticosteroid therapy for pro-found thrombocytopenia. There was a significant decrease in mean M-CSF level after treatment with corticosteroids in comparision to the pretreatment level of M-CSF (P < 0.05).

Table (3) showed the comparison between M-CSF level in patient with profound thrombocytopenia after corticosteroid therapy in comparison to the patient they did not receive corticosteroid therapy. There was no statistical significant difference between

the two groups ( $P > 0.05$ ).

Table (4) revealed the correlation coefficient ( $r$ ) and ( $p$ ) value between M-CSF levels and platelet count. There was a significant negative correlation between M-CSF levels and platelet count ( $r = -0.721$ ,  $P < 0.5$ ).

### RESULTS

Table (1) showed the mean of M-CSF level in 10 patients with thrombocytopenia before and after corticosteroid therapy. The mean M-CSF level before therapy was 1.2 ng/ml and after therapy was 1.1 ng/ml. There was no significant difference between the two groups ( $P > 0.05$ ). Table (2) showed the mean of M-CSF level in 10 patients with thrombocytopenia before and after corticosteroid therapy. The mean M-CSF level before therapy was 1.2 ng/ml and after therapy was 1.1 ng/ml. There was no significant difference between the two groups ( $P > 0.05$ ). Table (3) showed the comparison between M-CSF level in patient with profound thrombocytopenia after corticosteroid therapy in comparison to the patient they did not receive corticosteroid therapy. There was no statistical significant difference between the two groups ( $P > 0.05$ ). Table (4) revealed the correlation coefficient ( $r$ ) and ( $p$ ) value between M-CSF levels and platelet count. There was a significant negative correlation between M-CSF levels and platelet count ( $r = -0.721$ ,  $P < 0.5$ ).



**Table (1):** Mean  $\pm$  SD of M-CSF in the studied groups :

	Control (n = 25)	Patient (n = 25)	P
M-CSF (u/ml)	364 $\pm$ 69	929 $\pm$ 127	< 0.05

**Table (2):** Mean  $\pm$  SD of M-CSF in ITP patients before and after corticosteroid therapy.

	Patients before corticosteroid (n= 8)	Patients after corticosteroid (n= 8)	P
M-CSF (u/ml)	1235 $\pm$ 439	797 $\pm$ 402	< 0.01

**Table (3):** Comparison between the level of M-CSF in patients after corticosteroid therapy with the patients without therapy.

	Patients after corticosteroid (n= 8)	Patients without corticosteroid (n= 17)	P
M-CSF (u/ml)	797 $\pm$ 402	829 $\pm$ 321	> 0.05

**Table (4):** correlation coefficient (r) and (p) between M-CSF level and platelet count.

	r	P	Type of correlation
M-CSF (u/ml)	- 0.721	< 0.05	Strong negative correlation

## DISCUSSION

M-CSF is a hematopoietic growth factor that stimulates the growth, differentiation and proliferation of cells of monocyte-macrophage lineage and induces monocyte production of granulocyte colony stimulating factor (G-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), interferon, interleukin 1 and tumor necrosis factor (TNF).<sup>16</sup> M-CSF is produced by a variety of tissue and mesenchymal cells, including marrow stromal cells, fibroblasts and endothelial cells.<sup>17,18</sup>

In our study we have demonstrated that circulating M-CSF levels in children with ITP are significantly increased when compared with control children ( $P < 0.05$ ).

This could be explained by a fact that following exposure to viral infection, children will develop antibody against platelet surface. The exact antigen target for such antibodies in acute ITP remains undetermined. The antibodies bind to platelet surface, circulating antibody coated platelets are recognised by FC receptor on splenic macrophages, ingested and destroyed. Such receptor mediated interaction resulting in macrophage activation and the subsequent synthesis

and release of M-CSF, either by macrophages themselves or by other cells stimulated to produce M-CSF, which could lead to autocrine stimulation, thus increasing the destruction of antibody coated platelets.<sup>19</sup>

Georgiann et al.,<sup>20</sup> concluded that thrombocytopenia induced by intraperitoneal injection of mice with recombinant human (M-CSF) was not due to suppression of thrombopoiesis but to increased activity of monocyte/macrophage system, which caused shortened platelet survival.

Monocyte platelet interactions has been demonstrated in immune thrombocytopenic purpura patients and correlate well with the amount of platelet surface-bound IgG. such interaction may be responsible for macrophage activation.<sup>21</sup>

M-CSF has been reported to enhance the expression of both FcRI and FcRII on mature murine macrophages.<sup>22</sup>

A similar report has been obtained by Nomura et al<sup>11</sup>, who reported that the mean plasma level of M-CSF in adult patients with chronic ITP was significantly higher than in normal

controls. They reported a higher level of M-CSF in chronic adult ITP patients than in our acute children ITP patients.

Serum M-CSF levels are significantly elevated in adult non pregnant females with immune thrombocytopenia. The high level found in these patients may reflect the activation of mononuclear phagocytes, and could, in turn, result in increased platelet destruction.<sup>12</sup>. zeigler et al<sup>10</sup>., reported that, M-CSF levels were significantly higher in adult ITP patients than in the control subjects and they suggested that M-CSF may influence macrophage mediated platelet destruction in these patients.

In our study, there was a significant negative correlation between M-CSF and platelet count. This could be explained on the basis of, once macrophage activated and causes destruction of platelets, low platelet count could be accompanied with secretion of other growth factors. These growth factors could, in turn, stimulate M-CSF production by mononuclear cells, endothelial cells or T cells.

Cox et al.,<sup>23</sup> reported an increase in IL-6 mRNA following induction of

thrombocytopenia in mice by injecting recombinant M-CSF.

In our study, ITP patients with profound thrombocytopenia treated with corticosteroids, showed a significant reduction of plasma M-CSF levels in comparison to the pretreatment plasma M-CSF levels. These findings suggest that corticosteroids could induce thrombocytopenia by the decrease of M-CSF level in ITP patients.

Numura et al<sup>11</sup>., suggested that M-CSF levels decreased after intravenous gammaglobulin in adult patient with ITP. They demonstrated that high dose intravenous gammaglobulin also causes thrombocytopenia by decrease of M-CSF in adult patient with ITP.

These results suggest that M-CSF level may enhance macrophage activity and platelet destruction in ITP patients which may lead to a possible novel therapeutic approaches in a subset of patient with ITP.

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