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# Estimation of Serum Interferon-Gamma Level in Childhood Acute Lymphoblastic Leukemia Patients

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This study aimed to investigate the influence of acute lymphoblastic leukemia and the administration of chemotherapeutic treatment on serum interferon-gamma level. This study included 45 children of matched age, classified into 3 groups; group 1 was control group and included 15 normal healthy children, group 2 included 15 newly diagnosed acute lymphoblastic leukemia patients before receiving chemotherapy and group 3 included 15 acute lymphoblastic leukemia patients after receiving chemotherapy. Serum interferon-gamma showed a statistically significant increase in group 2 and group 3 as compared to group 1 ($p<0.0001$ and $p = 0.0017$, respectively). On the other hand, group 3 showed a statistically significant decrease compared to group 2 ($p<0.0001$). The present study revealed a significant effect for both acute lymphoblastic leukemia disease and the administration of chemotherapy on serum interferon-gamma level.

**Key words:** Interferon-gamma, IFN-γ, T lymphocyte, acute lymphoblastic leukemia, ALL, cytokine

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## INTRODUCTION

Acute Lymphoblastic Leukemia (ALL) is the most common cancer in children and represents about one quarter of all cancers among people younger than 15 years. ALL arises from genetic aberrations that impair the normal differentiation of lymphatic cells either at the stem cell or at an early lymphocyte precursor stage (Wu *et al*., 2005). These abnormal lymphocytes are not able to fight infection well. As the number of these lymphocytes increases, there is less room for healthy white blood cells, red blood cells and platelets. This may lead to infection, fatigue and easy bleeding (Zeller *et al*., 2007).

T lymphocytes are important for the host defense against infections and also as anti-leukaemic effector cells in patients with acute leukemia (Bruserud, 1998). The cells synthesize interferon-gamma (IFN-γ) and predominantly promote cell-mediated immune responses. In the last years, the importance of co-stimulatory molecules and activator molecules expression rises in immune response in leukemia (Luczyñski *et al*., 2004).

Interferons (IFNs) are a family of natural glycoprotein cytokines that share antiviral, immuno- modulatory and anti-proliferative effects. IFNs interact with specific cellular receptors and stimulate the production of second messengers, leading to the expression of antiviral and immunomodulatory proteins (Choi *et al*., 2004).

Clinically and experimentally, interferon-gamma has been shown to enhance the anti-tumor effects of anti-metabolite on cancer cells. Positive anti-tumor effects have also been obtained by immunotherapy with natural IFNs and interleukins, particularly in combination strategies. In tumor cell lines, IFN-γ can induce or modulate cell death either as a single agent or in combination with chemotherapeutic drugs. IFN-γ and IFN-α can up-regulate the expression of a number of apoptosis-related proteins in different types of cells (Varela *et al*., 2001).

Modern intensive chemotherapy has dramatically improved the prognosis of acute lymphoblastic leukemia in children. However, once remission has been established, quality of life and even survival may be threatened by exacerbation of viral infections in the prolonged period of continuation therapy necessary to prevent relapse (Nash *et al*., 1993).

The aim of this study is to investigate the influence of acute lymphoblastic leukemia and its chemotherapeutic treatment on serum interferon-gamma level.

## MATERIALS AND METHODS

**Subjects:** This study was carried out on 45 children attending National Cancer Institute in the period from January 2006 to April 2006. Their age ranged from 2 to 12 years.
They were classified into 3 groups:

**Group 1 ($G_1$):** Included 15 normal healthy children (10 males and 5 females).

**Group 2 ($G_2$):** Included 15 newly diagnosed patients with acute lymphoblastic leukemia (8 males and 7 females) before receiving chemotherapy.

**Group 3 ($G_3$):** Included 15 patients with acute lymphoblastic leukemia in remission after receiving chemotherapy (vincristine, prednisone/dexamethasone and L-asparaginase), they were 8 males and 7 females.

All children were subjected to full clinical examination and routine laboratory investigations; complete blood picture using automated cell coulter and liver function tests; alanine aminotransferase and aspartate aminotransferase. Patients were diagnosed by Bone Marrow (BM) aspiration and morphological examination of Leishman stained bone marrow films, cytochemistry and immuno-phenotyping.

**Immunological estimations:** Immuno-Phenotyping (IPT) by flow cytometer using monoclonal antibodies (Qadir *et al*., 2006) for all patients.

Estimation of serum interferon-gamma (IFN-γ) was done for all children using quantitative Enzyme-Linked Immunosorbent Assay (ELISA) technique (Biosource Kit).

**Statistical analysis:** Data were presented as mean±Standard Deviation (SD). Statistical analysis using SPSS program was performed and the comparison between groups of patients and controls were evaluated by the student's t-test. Differences were considered statistically significant when p-value was$<0.05$.

## RESULTS

Immuno-phenotyping of acute lymphoblastic leukemia patients included in this study revealed 60% of patients were common ALL, 33.3% were pre-B-ALL and 6.7% were T-ALL.

There were no statistically significant differences in serum alanine aminotransferase and aspartate aminotransferase between group 2 and group 3 ALL patients as compared to the control group (group 1).

Table 1: Laboratory characteristics for Group 1, 2 and 3

| Items | Group 1 (n = 15) | Group 2 (n = 15) | Group 3 (n = 15) | p-value comparing $G_1$ and $G_2$ | p-value comparing $G_1$ and $G_3$ | p-value comparing $G_2$ and $G_3$ |
|---|---|---|---|---|---|---|
| Hb (g $dL^{-1}$) | 12.420±0.44* | 6.800±1.41 | 9.500±1.1 | <0.0001 | <0.0001 | <0.0001 |
| TLC ($\times 10^9$ $L^{-1}$) | 7.670±1.53 | 18.600±5.89 | 5.490±2.75 | <0.0001 | 0.012 | <0.0001 |
| Platelets ($\times 10^9$ $L^{-1}$) | 219.670±36.08 | 99.330±25.76 | 165.330±31.05 | <0.0001 | 0.0002 | <0.0001 |
| IFN-γ (IU $mL^{-1}$) | 0.042±0.03 | 0.353±0.12 | 0.094±0.05 | <0.0001 | 0.0017 | <0.0001 |
| BM blast (%) | _ | 79.130±12.1 | 1.800±0.68 | _ | _ | <0.0001 |

*: Values are shown in (mean±SD)

From Table 1, it was noticed that there was statistically significant decrease in blood Hemoglobin (Hb) in group 2 and group 3 as compared to normal control group (group 1) ($p<0.0001$ in both).

Total Leukocyte Count (TLC) showed statistically significant increase in group 2 ($p<0.0001$) as compared to group I, while it showed a statistically significant decrease in group 3 ($p = 0.012$) as compared to group 1.

Blood platelets count showed a statistically significant decrease in group 2 and group 3 as compared to group 1 ($p<0.001$ and 0.0002, respectively).

Serum interferon-gamma showed a statistically significant increase in group 2 and group 3 as compared to group 1 ($p<0.0001$ and $p = 0.0017$, respectively).

The comparison between group 2 and group 3 showed statistically significant decrease in bone marrow blast in group 3 compared to group 2 ($p<0.0001$). Blood hemoglobin, TLC and blood platelets count showed statistically significant difference between group 2 and group 3. Serum interferon–gamma showed a statistically significant decrease in group 3 as compared to group 2 ($p<0.0001$).

All group 2 patients (100%) and 80% of group 3 patients showed increased serum interferon-gamma level than normal control group, also 73.3% of group 2 patients had blood Hb < 8 g $dL^{-1}$.

## DISCUSSION

Interferon (IFN) is crucial for initiating the innate immune response and for the generation of the adaptive response. IFN, in most species, comprises of IFN-α, IFN-β and IFN-γ (Yao *et al*., 2007). Interferons play key roles in mediating antiviral and antigrowth responses and in modulating immune response. The signaling pathways involve tyrosine phosphorylation and activation of signal transducers and activators of transcription factors at the cell membrane, followed by release of signal transducers and activators of transcription and their migration to the nucleus, where they induce the expression of the many gene products that determine the responses (Stark *et al*., 1998).

The anti-tumor properties of interferons against a variety of tumor cells such as lymphomas, melanomas and multiple myeloma had been demonstrated. Fujii *et al.* (2007) studied the immune activity against human acute lymphoblastic leukemia cells. He found that the death of leukemia cells *in vivo* was correlated with the production of IL-12, IFN-α and IFN- γ by the host.

In this study, we emphasized on serum IFN-γ, estimating its serum level in ALL patients before receiving treatment (group 2) and after administration of chemotherapy (group 3) compared to normal control group (group 1). We found statistical significant increase in serum IFN-γ in group 2 and group 3 as compared to group 1 (control group), also there was a statistically significant increase in serum IFN-γ in group 2 as compared to group 3, this was in agreement with Tuncer *et al*. (1996) study, they also suggested that short-course of high-dose of methylprednisolone given during treatment might be the cause of decreasing serum IFN-γ level in ALL patients after receiving chemotherapy. In agreement with our results, Ghosh *et al*. (2005) detected elevated level of serum interferon gamma in ALL children and that their peripheral blood mononuclear cells were continuously exposed to this cytokine. Luczyñski *et al*. (2004) demonstrated a rise in helper T lymphocytes producing IFN-γ (Th1), T cell activation and Th2 predominance at the time of diagnosis and during remission induction in ALL in children, which confirm the involvement of cellular immunity in the leukemic process and can be used in immune therapy in leukemia.

Ersvaer *et al*. (2007) investigated the release of IFN-γ and GM-CSF by circulating T cells in acute leukemia patients with chemotherapy-induced cytopenia, they found increased cytokine release during T cell activation, including the potentially anti-leukemic IFN-γ.

Yin *et al*. (2006) studied the intracellular IFN-γ level in ALL children, they found that the intracellular IFN-γ level was significantly lower in ALL patients than that in the control group and the intracellular IFN-γ level increased in ALL patients at remission stage, which was higher than that at diagnosis and the control group. In addition, they suggested that intracellular IFN-γ level may be used as a marker for monitoring the response to treatment in ALL patients. The same conclusion was noted by Park *et al*. (2006) on studying intracellular IFN-γ of leukemic cells and bone marrow T cells of acute leukemia patients, their cells were analyzed by flow cytometry and found IFN-gamma level was low in the

ALL group. Also, Zhang *et al.* (2000) studied intracellular interferon gamma production of their respective purified $CD^{4+}$ and $CD^{8+}$ T cells. They found IFN gamma-producing cell populations in $CD^{4+}$ and $CD^{8+}$ T cells of ALL patients decreased and the ability to produce IFN-γ was recovered after complete remission had been achieved. Dohnal *et al.* (2006) suggested that T-ALL may induce a specific cellular and humoral antileukemia immune response in children, thereby supporting new approaches for immunotherapy.

Present study showed significant decrease in blood hemoglobin in group 2 and group 3 as compared to group 1 and 73.3% of group 2 patients had Hb <8 g $dL^{-1}$, this was in agreement with study done by Settin *et al.* (2007) who revealed that 65% of ALL patients had Hb≤8 g $dL^{-1}$. Ek *et al.* (2005) studied plasma levels of interferon gamma in children with newly diagnosed cancer associated with hematopoietic suppression and whether hemoglobin level at diagnosis affects long-term prognosis in childhood acute lymphoblastic leukemia. They revealed that IFN-gamma was related to anemia in cancer patients. They also found that Hb levels were not associated with increased risk of ALL relapse. Felli *et al.* (2005) stated that IFN-gamma inhibits the growth and differentiation of erythroid precursor cells and mediates hemopoietic suppression.

In conclusion, the present study supported the involvement of interferon-gamma role in immune response of acute lymphoblastic leukemia patients and the influence of chemotherapy. We suggest further studies in exploring the potential role of immunotherapy in such patients and to design a specific immunotherapy for acute lymphoblastic leukemia patients.

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