Prognostic Impact of Elevated Serum Hyulronic Acid, Ferritin and Interleukin-6 in Patients with Acute Myeloid Leukemia

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Absract

Background: Acute myeloid leukemia (AML) is a clonal disease of hematopoiesis with poor clinical outcome despite recent improvements in chemotherapy and stem cell transplantation regimens. It is the most common acute leukemia in adults. Hyaluronic acid, ferritin and Interleukin-6 are involved in the pathogenesis of acute myeloid leukemia , but their prognostic significance in these diseases is unknown. In the current study, the authors assessed the serum levels of these parameters in different stages of the disease to predict their prognostic value, which might therefore represent interesting target for immunotherapy in patients with different hematological malignancies.
Methods: Serum levels of. hyaluronic acid, ferritin and Interleukin-6 were measured using a commercially available sandwich Enzyme Linked Immune Sorbent Assay (ELISA) kit in patients with AML who were attending for treatment at National Cancer Institute, Cairo University from September 2006 through January 2009.
Results: Newly diagnosed and relapsed patients with AML had significantly higher serum levels of hyaluronic acid, ferritin and Interleukin-6 compared with both control group and leukemic patients in remission stage. Serum levels of hyaluronic acid, ferritin and interleukin–6 in patients with AML (at diagnosis and at relapse) correlated inversely with the hemoglobin concentration. While their serum levels correlated positively with both total leukocyte count and with the % of blast cells in bone marrow in patients with AML

Conclusions: It could be concluded that serum levels of hyaluronic acid, ferritin and Interleukin-6 can be used as prognostic markers at diagnosis of adult AML and it could be used as follow up parameters for early detection of relapse. Furthermore, they might represent interesting target for immunotherapy in patients with different hematological malignancies. [Journal of American Science 2010;6(12):423-432]. (ISSN: 1545-1003).

Keywords: Acute myeloid leukemia (AML), Hyaluronic acid (HA), Ferritin (Fe), Interleukin–6 (IL-6).

1. Introduction:

Acute myeloid leukemia is a hematological disease characterized by the clonal proliferation of undifferentiated myeloid progenitor cells. AML is the most common variant of acute leukemia occurring in adults, comprising approximately 80 to 85% of cases of acute leukemia diagnosed in individuals greater than 20 years of age. Most of the patients with AML achieve a complete hematological remission by chemotherapeutical regimes. However, the long-term prognosis for all AML patients is rather poor with a 5-year overall survival of only 20-25% depending on the individual risk profile and the treatment option chosen. This clinical outcome suggests that the majority of the patients in complete hematological remission have minimal residual disease, subsequently leading to relapse. Obviously the leukemia-bearing host is immunologically tolerant to the remaining leukemia cells and therefore fails to eradicate the disease (**Li** *et al.,* **2003).**

Hyaluronic acid (HA) is a nonsulfated glycosaminoglycan (molecular weight of 10^6 dalton). It is the only glycosaminoglycan that is not attached to any protein core. It is a component of tissue matrix and tissue fluids, it maintains the cartilage integrity and osmotic balance. Also it regulates cell adhesion, migration and proliferation. It is produced mainly by fibroblasts and other specialized connective tissue cells. It plays a structural role as part of the connective tissue matrix (proteoglycan) and participates in various cell-to-cell interactions. Synovial hyaluronic acid passes into plasma via the lymphatic system, and is quickly removed from the blood by a receptor dependent mechanism in sinusoidal epithelial cells of the liver and by the enzymatic action of hyaluronidase . Thus, hyaluronan is a glucosaminoglycan synthesized by the mesenchymal cells and

degraded by hepatic sinusoidal endothelial cells by a specific receptor-mediated process **(Plevris *at al.* 2000 *)*.**

It has been reported that HA is synthesized mostly by tumor stromal fibroblasts and that tumor cells activate the fibroblasts to synthesize a high levels of hyaluronic acid .The concentration of HA is elevated in several carcinomas (e.g., lung, breast, colon, Wilms' tumor **(Delpech *et al*., 1997).** More importantly, it was shown that the HA concentration is elevated in the urine of bladder cancer patients and serves as a diagnostic marker for detecting bladder tumor regardless of its grade **(Lokeshwar *et al.*, 1997).**

Hyaluronan (HA) has been reported to bind specifically and with high affinity to various cell types and to directly modify cell behaviour. It was demonstrated that both high molecular weight molecules (HAH) and HA-derived oligosaccharides were efficient at triggering terminal differentiation of acute myeloid leukemia (AML) blasts, in vitro
(Marie *et al.,* 2004).

Iron-storage compounds in the body include hemoglobin, hemosiderin, myoglobulin and the cytochromes **(Adams, 1998).** In most tissues, ferritin is a major iron storage protein with a molecular weight of 45 kd. Each molecule contains as many as 4000 iron atoms. Ferritin represents 25% of the total iron found in the body. High concentrations of ferritin are found in the cytoplasm of the reticuloendothelial system, the liver, spleen and bone marrow. Methods previously used to measure iron in such tissues are invasive, cause patient trauma and lack adequate sensitivity. Ferritin levels in serum have been used to evaluate clinical conditions not related to iron storage, including inflammation, chronic liver disease and malignancy **(Casaril *et al.,* 2000).** In normal conditions ferritin is mainly expressed in red cell precursors and reticuloendothelial cells, and this is in keeping with the peculiar role of these cells in iron metabolism. Abnormal cell ferritin contents can be observed in both iron overload and malignancy. Elevated serum ferritin might indicate the presence of malignant disease . It was reported that serum ferritin was elevated in breast carcinoma, different urologic malignancies, and acute and chronic leukemia and in M1 and M2 myeloid leukemia **(Ulbrich *et al.,* 2003, Ahlawat *et al.,* 1994 and Aulbert *et al.,* 1991).**

Cytokines are involved in the pathogenesis of acute myeloid leukemia (AML) and high-risk myelodysplastic syndromes (MDS), but their prognostic significance in these diseases is unknown **(Tsimberidou *et al.*, 2008).**

Interleukin-6 (IL-6) is a pleiotropic cytokine produced by a variety of cell types, including fibroblasts, endothelial cells, monocytes, normal hematopoietic cells, and lymphocytes. Serum IL-6 levels have been correlated with an increased risk for development of lymphoma in patients with AIDS and in renal transplant recipients. Serum IL-6 levels are increased in diffuse large cell lymphomas, associated with adverse prognostic features, and predictive of a poor failure-free and overall survival in multivariate analysis. Interestingly, serum IL-6 levels may also be elevated and correlate with poor prognostic features and an inferior outcome in Hodgkin disease, indolent non-Hodgkin lymphomas (NHLs), renal cell carcinoma, prostatic cancer, ovarian cancer, and multiple myeloma (**Kawano *et al.,* 1988**).

2. Aim of the work:

The present study was planned to estimate the serum levels of hyaluronic acid, ferritin and interleukin-6 in patients with acute myeloid leukemia in different stages of the disease and to assess their prognostic value.

3. Subjects and Methods:

The subjects of this study were (115) divided into two groups:

Group A: Patients group consisted of 80 with AML attending the National Cancer Institute, Cairo University. They were 48 males and 32 females. They included 32 patients (group A I*),* at diagnosis before starting therapy with age range between 25 and 56 years (mean 44.2 ± 8.6). 28 patients in remission (group A II), but still under therapy with age range between 29 – 58 years (mean 47.5 ± 9.3)*.* and 20 patients (group A III),with bone marrow relapse with age range between 35 – 58 years (mean 48.3 ± 9.5).

Group B: (Control group) 35 healthy volunteers were used for comparison with AML patients, 20 males and 15 females with age range between 32 - 57 years (mean 45.7 ± 4.7). They were free from any acute or chronic disease (e.g., Liver dysfunction, diabetes, infection, etc.) at the time of samples withdrawal.

3.1. Methods

All patients included in this study were subjected to:

1- Full medical history with particular stress on age at diagnosis, sex, bone aches, neurological complications (for evidence of CNS involvement).

2 -Thorough clinical examination was done with special emphasis on the weight, height, temperature, lymph node enlargement,

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organomegaly, the presence of any mass as well as central nervous system and chest examination.

3 -Investigations included:

- Complete hemogram (using Automated Coulter Counter T-660).

- Examination of bone marrow aspiration and/or biopsy for morphology, FAB classification and immunophentyping done for the patients.

- Examination of CSF for evidence of CNS involvement.

- Radiological investigations included chest X-ray, bone survey, ultra-sonic and computed tomography scanning to chest and abdomen, and others as indicated.

3.2. Laboratory investigations:
All patients were subjected to :

3.2.1. Determination of serum Hyaluronic Acid:
Serum samples were analyzed for Hyaluronic Acid (HA) using a commercially available sandwich Enzyme Linked Immune Sorbent Assay (ELISA) kit obtained from Corgenix, inc., USA **(Chichibu *et al.,*1989).** HA levels in patients and control samples were determined against a reference curve. Each standard and sample was assayed in duplicate.

3.2.2. Determination of serum Ferritin:
Samples were analyzed for Ferritin using a commercially available sandwich ELISA kit obtained from DRG International inc., USA **(White *et al.,* 1986).** Each standard and sample was assayed in duplicate.

3.2.3. Determination of serum IL-6:
Analysis was performed using commercially available kit (IL-6 ELISA Kit), Diaclone Research, (URS), - France. The minimum detectable dose of IL-6 is less than 2 pg/ml. Intra and Inter - Assay coefficients of variation of the assay were 0.83-3.86% and 1.89-5.84% **(Robak *et al.,* 1999**).

4. Statistical analysis:
Data were analyzed with standard program of SPSS, Echo Soft corporation, USA, 1995 statistical package. Student t test was applied to the data conforming to normal distribution. For non-parametric data Mann Whitney U test was applied. Correlation coefficient (r) was used to determine the relationships between different quantitative values. For all tests a probability <0.05 was considered significant (**Saunders and Trapp 1995**).

5. Results:
The results of this study are demonstrated in the tables (1,2, and 3) and figures (1,2, and 3).

Table (1) shows descriptive data of the patients with acute myeloid leukemia and control groups. Newly diagnosed and relapsed AML patients had statistically significant lower hemoglobin and platelet count compared to control group ($p< 0.001$). TLC was statistically high among newly diagnosed and relapsed AML patients compared to control group and patients with AML at remission stage ($p < 0.001$). There was no significant difference between AML patients in remission and control group.

The results showed that newly diagnosed and relapsed patients with AML had significant high serum levels of hyaluronic acid, ferritin and interleukin–6 compared to both control group and leukemic patients in remission stage (Table 2).

There was no significant difference in serum levels of hyaluronic acid, ferritin and interleukin–6 between newly diagnosed and relapsed AML patients, as well as between leukemic patients in remission stage and control group, also there was no significant difference as regards FAB morphological classification and immunophenotype .

Serum levels of hyaluronic acid, ferritin and interleukin–6 in patients with AML (at diagnosis and at relapse) correlated inversely with the hemoglobin concentration ($p < 0.05$). While their serum levels correlated positively with both total leukocyte count (TLC) ($p < 0.05$) and with the % of blast cells in bone marrow in patients with AML.

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Table (1): Descriptive data of patients with Acute Myeloid Leukemia and control group.

Diagnosis	Age years	Hb gm%	WBCs X10^9/L	Plt X10^9/L	FAB Classification (n)
Group A I (n= 32) **At diagnosis** Range Mean SD	25-56 44.2 8.6	4.2-11.4 8.1 2.4	14-112 36.6 30.1	121-196 72.4 59.9	M2 = (10) M3 = (14) M4 = (8)
Group A II (n = 28) **In remission** Range Mean SD	29-58 47.5 9.3	8.5-13.3 10.2 1.7	1.9-8 5.8 1.9	74-320 196.6 2.32	M2 = (8) M3 = (11) M4 = (9)
Group A III (n =20) **In relapse** Range Mean SD	35–58 48.3 9.5	4.2–11.1 7.43 1.9	15–52 24.2 8.4	28–304 115.8 94.1	M2 = (6) M3 = (7) M4 = (7)
Group B (n = 35) **Controls** Range Mean SD	32–57 45.7 4.7	10–13 11.4 0.93	4.2–9.7 6.5 1.7	181–340 272 66.2	

n : number of patients   Hb : hemoglobin   Plt : platelet count

WBCs : white blood count   SD : standard deviation

Table (2): Serum levels of of Hyaluronic Acid, Ferritin, Interleukin 6 in the different studied groups of Acute Myeloid Leukemia and control group..

	Hyaluronic Acid (ng/ml)	Ferritin (ng/ml)	Interleukin – 6 pg/ml
AML group **Group A I (n= 32)** **At diagnosis** Range Median	65.62-286.87 184.33**■■	617.25-1875.35 1400.25**■■	0 - 64.23 24.83**■■
Group A II (n = 28) **In remission** Range Median	13.75-60.62 33.65	36.3 - 530.23 99.34	1.34 -18.34 8.24
Group A III (n =20) **In relapse** Range Median	70.73-286.22 188.98* *■■	749.25 -1896.54 1431.45**■■	3.41 - 65.12 19.32**■■
Group B (n = 35) **Controls** Range Median	9.37-55.26 23.18	8.86 - 98.23 36.69	0 -18.23 7.14

*   compared to control group (group B)
■   compared to leukemic patients at remission (group A II)
**■■   = highly significant p < 0.0001

Table (3): Correlation between Hyaluronic acid, Ferritin, Interleukin–6 and Hemoglobin concentration, Platelets count, White blood cells count and Blast cells in patients with AML at diagnosis and in relapse .

	Hyaluronic acid	Ferritin	Interleukin 6
Hb	r = -0.69∗∗	r = - 0.44 ∗	r = - 665∗∗
Platelets	r = -0.41	r = - 0.39	r =-0.40
White blood cells	r = 0.49∗	r = 0.69 ∗∗	r = 0.45∗
Blast cells	r = 0.52∗	r = 0.79 ∗∗	r = 0.49∗
Hyaluronic acid		r = 0.076	r = - 0.26
Ferritin			r = - 0.089

r =   correlation coefficient
∗   significant p < 0.05
∗∗   highly significant p < 0.01

Fig. (1) : Serum levels of Hyaluronic Acid (ng/ml) in the different studied groups of Acute Myeloid Leukemia.
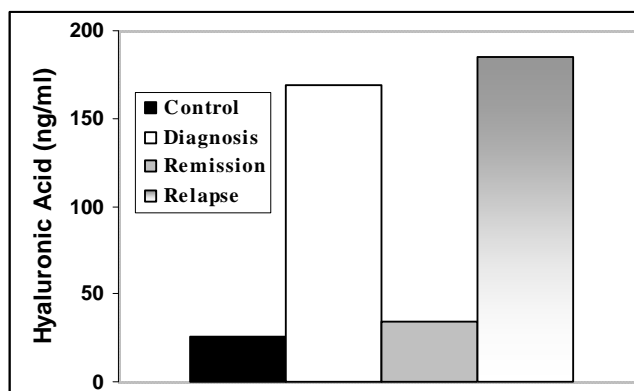

Fig. (2) : Serum levels of Ferritin (ng/ml) in the different studied groups of Acute Myeloid Leukemia.
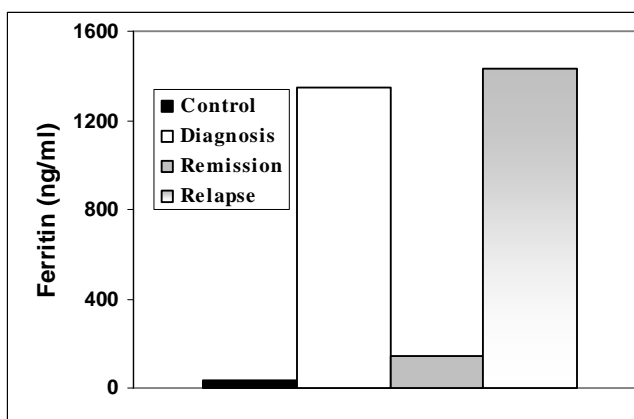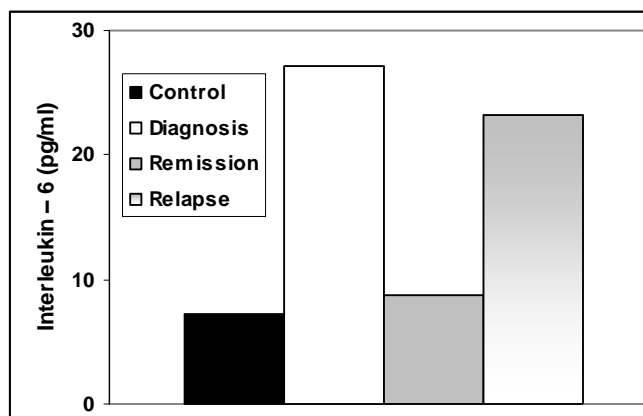
Fig. (3) : Serum levels of Interleukin – 6 (pg/ml) in the different studied groups of Acute Myeloid Leukemia

6. Discussion :

Acute myeloid leukemia (AML) is the most common acute leukemia in adults. With intensive induction therapy, most patients younger than 60 years achieve complete remission. However, even if these younger patients were treated intensively, more than 50% will relapse. Clinical results of patients older than 60 years are more unfavorable. Therefore, in all patients with AML, the overall survival is still low **(Tamm *et. al,* 2007).**

In our study, we demonstrated that adults with AML group (A) have higher plasma levels of hyaluronic acid, ferritin and Interleukin 6 when compared to control group. As regard Serum levels of hyaluronic acid, this study used an HABP based sandwich ELISA to show that serum HA level is raised in patients with AML in newly diagnosed and relapsed patients. In remission, serum levels of hyaluronic acid concentration decreased to the normal range. Hyaluronan (HA) has been reported to bind specifically and with high affinity to various cell types and to directly modify cell behaviour.

In a previous report **Courel *et. al,* 2004** demonstrated that both high molecular weight molecules (HAH) and HA-derived oligosaccharides were efficient at triggering terminal differentiation of acute myeloid leukemia (AML) blasts, in vitro. There have been a few reports of raised serum levels of HA in advanced cancer; **Delpech *et. al,* (1985)**. These studies confirm that a raised serum HA may accompany malignant disease. As yet, the mechanism of increased serum levels of HA in cancer is unclear. However, there are some clues from the case reports. Greatly increased levels of serum HA have been observed in Wilms' tumour **(Wu *et. al,* 1984; and Bracey**

***et. al,* 1987)** and neuroblastoma **(Pusch *et. al,* 2010).** In a few patients the rise of serum HA was associated with increased viscosity.

Our results agree with **Sanada *et. al,* 1999** who reported that levels of serum hyaluronic acid (HA) in adult T-cell leukemia (ATL) patients moved in parallel with the clinical activity of their disease. A hyaluronan-rich environment often correlate with tumor progression and may be there is one mechanism for the invasive behavior of malignancies. Eradication of hyaluronan by hyaluronidase administration could reduce tumor aggressiveness and would provide,therefore, a new anti-cancer strategy **(Adamia *et. al,* 2005).**

Giannopoulos *et. al,* 2009 reported that receptor for hyaluronic acid-mediated motility expression appears to be of prognostic value, as well as may reflect the proliferative capacity of chronic lymphocytic leukemia cells, and might therefore represent interesting target for immunotherapy in patients with different hematological malignancies **(Greiner *et. al,* 2010).**

In our study serum ferritin concentrations in patients with AML were found to increase in newly diagnosed and relapsed patients. This agree with findings of **Worwood *et. al,* 1974** who reported that the increased capacity for ferritin synthesis shown by myelogenous cells from patients with AML suggests that these cells themselves are the source of the increased amount of circulating protein. While patients during remission, the serum ferritin concentration decreased but still higher than the normal range. **White *et. al,* 1974** have shown that the increase in cirulating ferritin during chemotherapy could be due to an increased release from damaged leukaemic cells and this increase in serum ferritin

concentration was not correlated with the amount of blood transfused or the degree of liver damage .

Similar results were obtained by **Garcia-Manero in (2008)**and confirm the previous study of serum ferritin concentrations in acute leukaemia**.** It shows that serum ferritin is a marker of acute phase reactions and iron storage. In addition, hematologic malignancies are associated with elevated serum ferritin levels. There are a number of factors which are likely to contribute to raised serum ferritin concentrations in leukaemia: (1) Most of the patients are anaemic and have increased amounts of storage ferritin which are reflected by increased serum ferritin concentrations. (2) Increased synthesis of ferritin in the large mass of leukaemic cells is also reflected in high serum ferritin concentrations. In the earlier study, there was a significant correlation between leucocyte and serum ferritin concentration. (3) Abnormal release of ferritin from damaged cells is another possible cause of high serum ferritin concentrations **(Cragg et. al, 1977).**

Data from 90 patients with a variety of hematologic malignant neoplasms were studied by **Patel et. al, 1980** to determine the relation between changes in serum ferritin concentration and the clinical status of the patients. Patients with Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma, blastic crisis of chronic myelocytic leukemia, acute myeloblastic leukemia and acute lymphoblastic leukemia were found to have significantly elevated serum ferritin levels. The serum ferritin level reflects acute phase reactions and is usually associated with iron storage. Iron overload increases the susceptibility to organ damage and the risk for infection **(Miceli et. al, 2006).** Other recent studies have suggested that ferritin is a surrogate for advanced disease and has an impact on relapse, because elevated serum ferritin predicts overall survival (OS) and relapse-free survival following autologous stem cell transplantation for lymphomas **(Armand et. al, 2007, Mahindra et. al, 2008 and Moo-Kon Song et. al, 2009) .**

However, **Patel et. al,** in their study in **(1980)** showed that patients with hematologic malignancies have significantly higher serum ferritin levels. Another study showed that the intensity of the 99 mTc-MIBI scan correlates with the serum ferritin level as a marker of disease activity **(Alexandrakis et. al, 2001). Papadaki et. al,** in their study in **(1997)** found that elevated serum sIL-6R levels were related to the growth of myeloma cells and that the

concentration was an indicator of disease activity; the sIL-6R level was correlated with the serum ferritin concentrations as a marker of disease activity. However, the low serum ferritin concentrations found in patients with AML in remission suggest that ferritin concentration may be a useful index for the prediction of relapse and as a prognostic sign.

Several cytokines have been shown to promote the growth of malignant cells in vitro and are therefore believed to contribute to the aggressiveness of the disease **(Tsimberidou et. al, 2008).** In our study increased serum IL 6 concentrations were found in newly diagnosed and relapsed patients with AML. In remission, serum IL 6 concentration fall within the normal range. Several studies by **Lauta, 2003, Sohara et. al, 2005,and Hong et. al, 2007** reported that IL-6 is a pleiotropic cytokine with many ascribed effects including stimulation of acute phase reactants, immune regulation, angiogenesis, and osteoclast activation, and it originates from a multitude of cell types, including mononuclear phagocytes, vascular endothelial cells, fibroblasts, hepatocytes, B-cell lymphomas, and the neoplastic plasma cells of multiple myeloma. It appears to serve as a stimulatory factor in multiple myeloma; produced by both the malignant cells and bone marrow stromal cells**(Lauta, 2003).**

Our results agreed with **Tsimberidou et. al, 2008** who concluded that interleukin-6 (IL-6) may play a relevant role in the pathogenesis of several hematologic malignancies. IL-6 has diverse effects on the growth of AML blasts, including stimulation and maintenance of their growth through the IL-6/ IL-6 receptor signaling system. Also our results agreed with **Thomas et. al, 1997** who reported that AML relapse is suggested to result from treatment failure due to leukemic cells being resistant to chemotherapy and/or escaping immune surveillance. Due to the association of IL-6 expression with disease progression reported in previous studies, and it is conceivable that IL-6 may play an active role in relapse of ALL by supporting chemoresistance and inhibition of immunocompetent cells. Also they concluded that serum levels of IL-6 are a powerful prognostic factor in diffuse large cell lymphoma and chronic lymphocytic leukemia.

In the current study serum levels of hyaluronic acid, ferritin and interleukin–6 in patients with AML (at diagnosis and at relapse) correlated inversely with the hemoglobin concentration ($p < 0.05$). While their serum levels correlated positively with both total leukocyte count (TLC) ($p < 0.05$)

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and with the % of blast cells in bone marrow in patients with AML. So, these parameters may be useful and sensitive tumour markers.

Our results agree with **Sanada et. al, 1999** who reported that levels of serum hyaluronic acid (HA) in adult T-cell leukemia (ATL) patients moved in parallel with the clinical activity of their disease. **Papadaki et. al, 1997** concluded that the sIL-6R level was correlated with the serum ferritin concentrations as a marker of disease activity. Also they reported that low serum ferritin concentrations found in patients with AML in remission suggest that ferritin concentration may be also a useful index for the prediction of relapse and as a prognostic sign. This also agree with **Tsimberidou et. al, 2008** who found that IL-6 correlated inversely with Hb % and positively with the absolute number of circulating myeloblasts and the proportion of bone marrow myeloblasts in patients with high risk AML. Also **Preti et. al, 1997 and Fayad et. al, 2001** reported that serum levels of IL-6 are a powerful prognostic factor in diffuse large cell lymphoma and chronic lymphocytic leukemia. Also multiple studies have been done to explain the role for elevated IL-6 levels at diagnosis as a marker of poor prognosis in various cancers including multiple myeloma (**Lauta, 2003),** malignant melanoma (**Mouawad et. al, 2002 and Soubrane et. al, 2005**), non-Hodgkin's lymphoma (**El-Far et. al, 2004 and Pedersen et. al, 2005**), prostate cancer (**George et. al, 2005**), squamous cell carcinoma of the head and neck (**Riedel et. al, 2005**), and various sarcomas (**Rutkowski et. al, 2002**).

In conclusion, our data suggest that serum levels of hyaluronic acid, ferritin and interleukin–6 can be used as prognostic serum markers at diagnosis of adult acute myeloid leukemia and it could be used as follow up parameters for early detection of relapse.Understanding their roles may therefore represent interesting target for designing new therapeutic strategies for patients with different hematological malignancies.

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