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# Assessment of Serum Hyaluronic acid and Ferritin levels in children with Acute Lymphoblastic Leukemia

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## Abstract:

**Background:** Acute lymphoblastic leukaemia is the most common form of cancer (25–30%) and predominant subtype of leukaemia (75–80%) in children. It represents a malignant expansion of immature lymphoid cells that results from multi-step genetic changes in a single lymphoid progenitor cell. Hyaluronic acid is a nonsulfated glycosaminoglycan. It is found mainly in the extracellular matrix (ECM), especially of the loose connective tissue, including the bone marrow. HA influences many processes such as angiogenesis and the growth and migration of cells. HA levels are elevated in various cancers including AML, lung and breast cancers but their prognostic significance is unclear. Ferritin is the primary iron storage protein. High concentrations of ferritin are found in the cytoplasm of the reticuloendothelial system;the liver, spleen and bone marrow. It is also found in extracellular compartments, such as the serum. Serum ferritin is significantly affected by acute and chronic inflammation and is frequently increased at diagnosis in several cancers. The aim was to to evaluate the level of serum hyaluronic acid and ferritin in patients with ALL at diagnosis and in remmision, to correlate with hematological,biochemical and clinical parameters and to investigate if serum HA and ferritin could have a prognostic impact on disease development. **Materials and Methods:** A total of 60 subjects were enrolled in this study, 40 patients of ALL as cases (20 patients at diagnosis before starting chemotherapy and 20 patients in remission but still under therapy); and 20 age and gender-matched healthy subjects as controls. Serum HA was measured using a commercially available sandwich ELISA kit while serum ferritin was measured by immunoassay using an automated clinical chemistry analyzer. **Results:** Serum HA in newly diagnosed patients with ALL was significantly higher than either patients in remission or controls which suggests that HA could have a role in the malignant process of ALL.
Both patient groups (newly diagnosed and those in remission) had elevated levels of serum ferritin with no significant difference. This could be due to infection, inflammation or release of ferritin from damaged leukemic cells and other ferritin containing cells during chemotherapy. Serum levels of HA correlated positively with both serum ferritin and LDH. Conclusion: Our data suggest that serum level of hyaluronic acid could serve as a possible prognostic marker in ALL. Also it may represent an interesting target for new therapeutic strategies.

***Keywords:*** *ALL, Hyaluronic acid, Ferritin*

## Introduction:

Acute lymphoblastic leukemia (ALL) is a neoplastic disease that results from multistep somatic mutations in a single lymphoid progenitor cell at one of several discrete stages of development. Proliferation and accumulation of clonal blast cells in the marrow result in suppression of hematopoiesis and, thereafter, anemia, thrombocytopenia, and neutropenia. Lymphoblasts can accumulate in various extramedullary sites, especially the meninges, gonads, thymus, liver, spleen, and lymph nodes(1). Only a small proportion (<5%) of patients with childhood ALL have underlying hereditary genetic abnormalities. Children with Down syndrome have a10-to 30-fold increased risk of developing ALL (2).Familial disorders of DNA repair have been implicated in the induction of ALL in some patients. Environmental agents, such as ionizing radiation and chemical mutagens may play a role. However, in most cases, no etiologic factors are discernible. In the favored theory, leukemogenesis reflects the interaction between host pharmacogenetics (susceptibility) and environmental factors(1).Acute lymphoblastic leukaemia is the most common form of cancer (25–30%) and predominant subtype of leukaemia (75–80%) in children (2). It is more common in males than in females and in the USA,

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childhood ALL is considerably less common in Black Americans than in Whites(3). According to the Iraqi Cancer Registry 2015; leukemia had the highest incidence of cancer in children (3/100000 children 0-14 years population) (4).

**Hyaluronic acid** is a nonsulfated glycosaminoglycan. It is the only glycosaminoglycan that is not attached to any protein core. It consists of repeated disaccharide units which are composed of D-glucuronic acid and D-N-acetylglucosamin . It is found mainly in the extracellular matrix (ECM), especially of the loose connective tissue, including the bone marrow(5,6). As an integral component of ECM, HA influences many processes such as embryogenesis, angiogenesis, wound-healing, and the growth and migration of cells. It also plays a crucial role in the microenvironment, coregulating cell behavior during inflammation, cancer , and tumorigenesis (7)

**Ferritin** is the primary iron storage protein and provides a reserve of iron. Ferritin consists of a spherical protein (molecular mass 480 000 Da) enclosing a core of ferric-hydroxy-phosphate, which may contain up to 4000 atoms of iron. Human ferritin is made up from 24 subunits of two immunologically distinct types:H and L.There are multiple gene copies, which are mostly pseudogenes(8,9). Serum ferritin correlates roughly with total body iron stores, significantly affected by acute and chronic inflammation and is frequently increased at diagnosis in several cancers.

**Patients, Materials and Methods** This prospective cross sectional study was conducted in the Central Teaching Hospital of Paediatrics, Baghdad, Iraq from January 2020 to October 2020 .

A total of 60 subjects were enrolled in this study, 40 patients of ALL as cases (20 patients at diagnosis before starting chemotherapy and 20 patients in remission but still under therapy); and 20 age and gender-matched healthy subjects as controls.

The diagnosis of ALL was based on morphology ,cytochemistry of the peripheral blood and/or bone marrow aspirate smears by expert hematologist and immunophenotyping.

Four millileters of venous blood were collected from each participant with aseptic precautions from the antecubital vein. The collected blood was put in the refrigerator at 4 C° for the night. Then was centrifuged for 10min at 1000-3000rpm. The serum was taken and the sample was stored at -80 C° for 3 months. Serum HA was measured using a commercially available sandwich ELISA kit while serum ferritin was measured by immunoassay using an automated clinical chemistry analyzer.

**Inclusion and exclusion criteria**

All patients were diagnosed with ALL, both B- and T-ALL subtypes were included and the age was less than 15 years. The patients were randomly selected in relation to gender.

The exclusion criteria included patients on iron supplementation or on drugs affecting iron metabolism, patients with hepatitis or other liver diseases, patients with other type of malignancy and patients with skin or rheumatological diseases.

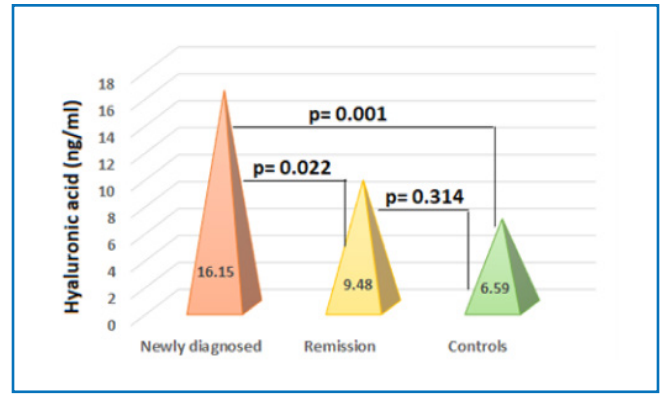
**Statistical Analysis**

All statistical analyses were performed using SPSS statistical software, version 25 (IBM Corporation, USA). Quantitative variables were presented as mean ± standard deviation (SD) and compared by student t-test when there were two groups, or analysis of variance (ANOVA) when there were more than two groups. Categorical variables were expressed as counts and percentages and analyzed with Chi square. Receiver operating characteristic curve was used to find out the diagnostic value of HA and LDH in discrimination between newly diagnosed patients and those with remission. Correlations between HA, ferritin and LDH with other variables were performed with two-tailed Pearson's correlation analysis. For all tests, a significant level of statistics was considered when $p<0.05$.

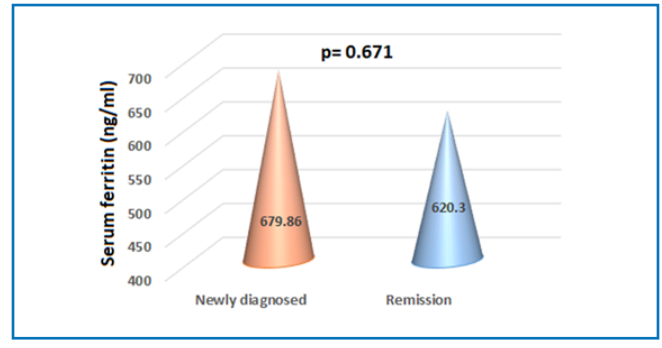
## Results:

Most of patients were in the age class of 1-10 years and male:female ratio was 1.5:1. The vast majority of patients were anemic at diagnosis. More than half of the patients had WBC count ≤ 50 ×109/L and 80% of them had a platelet count of ≤100 ×109/L. T-ALL type was encountered in 5 patients (25%).

Newly diagnosed patients showed higher serum concentration of HA (mean= 16.15± 6.52 ng/ml, range 2.55-40.79 ng/ml) than either patients at remission (mean= 9.48± 3.89 ng/ml, range 0.211-27.32 ng/ml) or controls (mean= 6.59± 2.88 ng/ml, range 0.59-19.34 ng/ml) with highly significant differences. There was no significant difference between remission group and controls (Figure 1)




Newly diagnosed patients demonstrated slightly higher serum concentration of ferritin than remission group (mean= 697.86±190.1 ng/ml, range 93.2-1500 ng/ml versus mean= 620.3±184 ng/ml, range 250-1500 ng/ml). However, the difference was not a significant (Figure 2).In general all patients (newly diagnosed and those in remission) displayed ferritin levels higher than the normal limits ( 7-140 ng/ml ). HA showed a



significant positive correlation with LDH (r= 0.551, p<0.001) and ferritin (r= 0.670, p<0.001) as shown in table 1.

| Variable | HA | | Ferritin | | LDH | |
|---|---|---|---|---|---|---|
| | r | p-value | r | p-value | r | p-value |
| Age | 0.037 | 0.822 | 0.004 | 0.980 | 0.174 | 0.327 |
| Hemoglobin | 0.069 | 0.773 | -0.04 | 0.867 | 0.348 | 0.133 |
| Platelets | -0.193 | 0.415 | -0.247 | 0.294 | -0.025 | 0.918 |
| WBC | -0.104 | 0.663 | -0.380 | 0.099 | 0.315 | 0.176 |
| Blast | 0.081 | 0.734 | -0.167 | 0.480 | 0.295 | 0.207 |
| LDH | **0.551** | **0.001>** | 0.159 | 0.327 | | |
| Ferritin | **0.670** | **0.001>** | | | | |

## Discussion:

Serum concentration of HA was higher than either patients in remission or controls with highly significant differences and this is in agreement with Anagnostopoulou et al (5). In earlier studies, considerably elevated serum HA values have been observed in AML, multiple myeloma, lymphoma , myelofibrosis, and CML , and also in various nonhematological diseases (5,10,11) . These studies confirmed that elevated serum HA levels may accompany malignant diseases. It is synthesized and secreted by fibroblasts and endothelial cells, catabolized by local degradation or carried away from the bone marrow via the blood circulation.Alterations in the hepatic blood or lymph flow may influence the kinetics of HA elimination.(5) Also, It has been reported that HA is synthesized by tumor stromal fibroblasts and that tumor cells activate the synthesis of high levels of hyaluronic acid by the fibroblasts .(12) Anagnostopoulou et al reported that the concentrations of serum HA in patients with acute leukemia (AML and B-ALL) on D0 who did not survive were much higher than in patients who survived. Giannopoulos et. al, 2009 reported that a receptor for hyaluronic acid-mediated motility expression appears to be of prognostic value, and may reflect the proliferative capacity of chronic lymphocytic leukemia cells, and therefore it may represent an interesting target for immunotherapy in patients with different hematological malignancies (13).

So the increased levels of serum hyaluronic acid in newly diagnosed ALL patients could be due to increased synthesis by the stromal fibroblasts under the effect of tumour cells, or decreased HA elimination due to alterations in hepatic blood or lymph flow.

In this study, there is a significant increase in serum ferritin levels among patients with acute lymphoblastic leukemia when compared with normal levels for age and this is an agreement with Anagnostopoulou et al(5) and Hamad et al(14).In our study newly diagnosed patients demonstrated slightly higher serum concentration of ferritin than remission group .Ferritin is a cellular storage protein for iron.It belongs to the acute-phase reactants, and it accompanies both inflammation and tissue injury. Several studies had reported that patients with elevated ferritin levels have a worse prognosis than those with lower or normal levels (12,15) . In addition many other studies reported that serum ferritin is significantly increased in AML and other hematological malignancies, with important prognostic implications .(12,16-18) Garcia-Manero et al. (15) reported that, in patients with acute leukemia, serum ferritin is a marker of acute-phase reactions and iron storage. Possible factors that are likely to contribute for raised serum ferritin levels in ALL patients in remission could be infection, iron overload, and chemotherapy.

The serum concentration of LDH is significantly increased in newly diagnosed patients with ALL and is much higher than remission group. This is in agreement with Anagnostopoulou et al(5), Hamad, et al(14) and many other studies. It is a well-known poor prognostic factor in patients with malignancies and it is frequently elevated in acute leukemia patients , reflecting malignant cell proliferation.(12,19). LDH enzyme is a very sensitive indicator of the cellular metabolic state, aerobic or anaerobic direction of glycolysis, activation status and malignant transformation.(20). Tumor burden, reflected by serum lactate dehydrogenase (LDH) level, initial white blood cell count (WBC), tumor size, and extensive bone marrow involvement are the main predictors for development of tumour lysis syndrome patients with acute leukemia.(21)

In our study, HA showed a significant positive correlation with LDH (r= 0.551, p<0.001) and ferritin (r= 0.670, p<0.001) and this is in agreement with Anagnostopoulou et al and Maksoud

et al for AML. This positive correlation may imply a relationship between HA metabolism and the activity of the malignant process

**Conclusion**

Our data suggest that HA could have a role in the malignant process of ALL as children with newly diagnosed ALL showed higher serum concentration of HA than either patients at remission or control cases with highly significant differences. ALL patients have raised serum ferritin levels. HA showed a significant positive correlation with ferritin and LDH. Thus, we suggest that serum HA may serve as a possible marker for aggressiveness in ALL patients, particularly before the beginning of the chemotherapy.

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**Conflicts of interest**

There are no conflicts of interest.

## References:

1. Larson RA. Acute lymphoblastic leukemia. In: Kaushansky K, Lichtman MA, Prchal JT et al. Wiliams Hematology. 9th ed. China. The McGraw-Hill education. 2016; 91: 1505-1527.
2. Vora A. Childhood acute lymphoblastic leukemia. In: Hoffbrand AV, editor. Postgraduate Hematology. 7th edition. UK: Wiley Blackwell publishing 2016;22:384-98.
3. Bain BJ. Acute Lymphoblastic Leukaemia and Acute Leukaemia of Ambiguous Lineage. In: Bain BJ, editor. Leukaemia diagnosis. 5th ed. London UK: Wiley Blackwell; 2017.P.250-294.
4. Iraqi Cancer Registry Ministry of Health, Iraqi Cancer Board, Baghdad, Iraq;2015.
5. Anagnostopoulou E,Papastamataki.Serum hyaluronic acid levels are altered in acute leukemia patients:potential prognostic implications.Acta Hematol 2017;138:44-51.
6. Petra Seebeck, and Peter Haima: Hyaluronic Acid (Hyaluronan) Biomarker for liver fibrosis and cirrhosis, joint disease, inflammation and others. TECOmedical Clinical & Technical Review August 2013.
7. Uchakina ON, Ban H, McKallip RJ: Targeting hyaluronic acid production for the treatment of leukemia: treatment with 4-methylumbelliferone leads to induction of MAPK-mediated apoptosis in K562 leukemia. Leuk Res 2013; 37: 1294–1301.
8. Clara Camaschella. Iron metabolism, iron deficiency and disorders of haem synthesis. In: Hoffbrand AV, editor. Postgraduate Hematology. 7th edition. UK: Wiley Blackwell publishing 2016; 3:21-39.
9. Mark Worwood. Iron Deficiency Anaemia and Iron Overload.In: Dacie and Lewis Practical Haematology. 12th edition. 2017; 9:165-189.
10. Liang J, Jiang D, Noble PW: Hyaluronan as a therapeutic target in human diseases. Adv Drug Deliv Rev 2016;97:186–203.
11. Jinfen Wei, Meiling Hu, Kaitang Huang: Roles of Proteoglycans and Glycosaminoglycans in Cancer Development and Progression. International Journal of Molecular Sciences August 2020.
12. Nabila Abd El Maksoud,Hala M. Ragab:Prognostic impact of elevated serum hyaluronic acid,ferritin and interleukin-6 in patients with acute myeloid leukemia.Journal of American Science 2010;6(12).
13. Giannopoulos K., Mertens D., Bühler A., Barth T. , Idler I., Möller P. and Kröber A. The candidate immunotherapeutical target, the receptor for hyaluronic acid-mediated motility, is associated with proliferation and shows prognostic value in B-cell chronic lymphocytic leukemiaRHAMM as immunotarget and prognostic marker in CLL Leukemia; 2009, 23, 519-527.
14. Mosab N. M Hamad, Mogdad Kamal , Mohamed A Saeed and Mazin A Suliman. Assessment of Serum Ferritin Levels in Sudanese Patients with Acute Lymphoblastic Leukemia. International Journal of Medical Research & Health Sciences, 2019, 8(7): 92-96.
15. Garcia-Manero G, Shan J, Faderl S, Cortes J, Ravandi F, Borthakur G, Wierda WG, Pierce S, Estey E, Liu J, Huang X, Kantarjian H: A prognostic score for patients with lower risk myelodysplastic syndrome. Leukemia 2008; 22:538–543.
16. Takayoshi Tachibana, Taiki Andou, Masatsugu Tanaka. Clinical significance of serum ferritin at diagnosis in patients with acute myeloid leukemia. Clinical Lymphoma, Myeloma and Leukemia;2018.
17. Sarah Bertoli , Etienne Paubelle , Emilie Bérard. Ferritin heavy/light chain (F TH1/F TL) expression, serum ferritin levels and their functional as well as prognostic roles in acute myeloid leukemia. European Journal of Haematology;Feb 2019.
18. Jana Ihlow, Sophia Gross, Annabel Sick, Tanja Schneider. AML: high serum ferritin at initial diagnosis has a negative impact on long-term survival. LEUKEMIA & LYMPHOMA, 2018.
19. Liu R, Cao J, Gao X, Zhang J, Wang L, Wang B, Guo L, Hu X, Wang Z: Overall survival of cancer patients with serum lactate dehydrogenase greater than 1,000 IU/L. Tumour Biol 2016;37:14083–14088
20. Jurisic, Vladimir, Sandra Radenkovic, and Gordana Konjevic. "The actual role of LDH as tumor marker, biochemical and clinical aspects." Advances in Cancer Biomarkers. Springer, Dordrecht, 2015. 115-124.
21. Belay, Yohannes, Ketsela Yirdaw, and Bamlaku Enawgaw. "Tumor lysis syndrome in patients with hematological malignancies." Journal of oncology 2017 (2017).