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Etiopathogenesis of Cancer-Related Anemia among Children and Adolescents with Malignant Solid Tumors

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Abstract

Background: Anemia in cancer patients is a common co-morbidity at diagnosis and throughout treatment, which does not appear to be a consequence of concurrent antineoplastic therapy, rather than being multifactorial. Anorexia, Malnutrition and lack of specific components (such as iron, vitamins, folic acid, etc.) fundamental for erythropoiesis contribute to the multifactorial pathogenesis of anemia. Caner related anemia (CRA) is primarily caused by the chronic inflammation associated with the cancer pathogenesis, as cancer cells mediate the activation of immune cells particularly the macrophages increasing the secretion of interleukin-6 (IL-6) among other inflammatory mediators contributing to CRA.

Aim of Study: To evaluate IL 6 levels among children and adolescents presenting with suspected solid tumors induced CRA, as well as recognition of iron status and other possible causes of anemia in pediatric malignant solid tumors.

Patients and Methods: A cross sectional study included 30 patients with solid tumors and anemia following at hematology/oncology clinic, Ain Shams University. Detailed review of the patient's clinical and laboratory data of disease diagnosis, stage at diagnosis, daily iron intake, bone marrow biopsy results, and IL6 assay by ELISA, as well as serum ferritin, transferrin saturation index (TSI) were done upon anemia presentation.

Results: Thirty patients with Solid Tumors were recruited with median (IQR) age of 6(2.5-10) years at presentation, 26.7% (n=8) diagnosed with brain tumors and 23.3% (n=7) with neuroblastoma. The Mean±SD hemoglobin was 9.21±1.33 g/dL, among which 80% (n=24) showed inadequate 24 hrs. iron intake. Nineteen patients (63.3%) had functional iron deficiency anemia, while 36.7% (n=11) hadcombinedtrue and functional iron deficiency anemia. IL 6 median (IQR) level=88.6pg/mL (57.5-156.5), which showed no statistically significant difference (p=0.561) among patients with true and functional iron deficiency.

Conclusion: Anemia among cancer patients can be multifactorial with some of patient's experiencing true iron deficiency anemia that can be managed by proper dietetic iron supplementation, Yet CRA represents a state of chronic inflammation with functional iron deficiency that needs proper recognition.

Key Words: *Cancer – Anemia.*

Introduction

CANCER-RELATED anemia (CRA) is classically normochromic and normocytic anemia with reticulo-cytopenia **[1]**. In 30 to 60% of the cancer patients despite associating iron deficiency, serum iron values are within normal range for age together with decreased total iron binding capacity (TIBC), as well as elevated serum ferritin level and transferrin saturation index (TSI) **[2]**. The pathogenesis of anemia in cancer patients is multifactorial, which can be attributed to either direct tumor cell bone marrow infiltration, cancer therapy owing to the myelosuppressive effects of the chemotherapy either alone or in combination with radiotherapy, and sometimes due to nutritional deficiencies **[3]**. Cancer related anemia (CRA) is now recognized as an immune mediated disorder as a consequence of tumor cell population interaction with the immune system; resulting in exaggerated expression of specific inflammatory cytokines with the resultant shortened red blood cells (RBCs) survival, depression of erythroid precursors, defective iron utilization, and impaired erythropoietin production and function **[4]**. This inflammatory dys-regulation results in the development of the true CRA via suppression of the medullary erythroid precursor, decreasing erythropoietin (EPO) hormone production as well as impairing body iron metabolism **[5]**.

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Cancer cells mediates the activation of the macrophages and with the secretion of interleukins primarily interleukin 6 (IL-6), as well as secretion of the tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ) **[6]**. Knowledge gap exists about CRA in pediatric cancer patients, which urged our research in the immuno-pathogenesis background of CRA and the role of IL6 and body iron status in determining patients'hemoglobin (Hb) status.

Patients and Methods

Study design:

In a cross-sectional study included 30 anemia patients diagnosed with brain and solid tumors following at the hematology/oncology clinic, Ain Shams University from Dec 2022 till December 2023 and excluding patients with gastero-intestinal and patients with relapsed/refractory brain or solid tumor. Patients were subdivided into: Group I+II: Patients with absolute iron deficiency anemia (AIDA) and Patients with combined absolute and functional iron deficiency anemia (CID) (n=11), Group III+ IV: Patients with functional iron deficiency anemia (FID), and patients with transferrin more than 20 and ferritin was more than 100 anaemia not related to iron (n=19). The Median (IQR) age of the patients was 6 (2.5-10) years. The study was approved by the Research Ethics Committee of the Faculty of medicine, Ain Shams University under acceptance number FMASU MS 320/ 2022 and are in accordance with the Helsinki Declaration of 2013. The details of the study and procedures were explained to all patients and their legal guardians, and informed consent and/or assent was obtained from the legal guardians and patients whenever possible before enrollment in the study after being informed of the study objectives and ensuring data confidentiality. The patients sub-grouping was carried outaccording to the WHO definition and grading of anemia according to age and gender (7) (Table 1). Based upon iron status, patients were classified into absolute iron deficiency (AID), functional iron deficiency (FID) or combined Fe deficiency (CID) anemia **[8]** (Table 2).

Table (1): WHO classification of anemia according to age and severity **[7]**.

Population age	Non-anemia	Mild anemia	Moderate anemia	Severe anemia
6-59 months	≥11	10-10.9	7-9.9	<7
5-11 years	≥11.5	11-11.4	8-10.9	<8
12-14 years	≥12	11-11.9	8-10.9	<8
Non pregnant women (15 years)	≥12	11-11.9	8-10.9	<8
Pregnant women	≥11	10-10.9	7-9.9	<7
Men 15 years	≥13	11-12.9	8-10.9	<8

Table (2): Anemia classification according to Fe status **[8]**.

	AIDA	FIDA	CIDA
Serum ferritin (ng/ml)	<30	>100	30-100
TSI (%)	<20	20-50	<20

AIDA = Absolute Fe deficiency anemia.
FIDA = Functional Fe deficiency anemia.
CIDA = Combined Fe deficiency anemia.
TSI = Transferrin situation index.

Data collection:

Data were collected from the patients' medical records and included demographic data,socio economic status according to the Egyptian based score by El-Gilany et al., **[9]** diagnosis, treatment received, previous history of bleeding, operations and transfusion.

Procedure:

Peripheral blood samples were withdrawn from all study patients and subjected to:

1- Complete blood cell count (CBC) using Sysmex XN-1000 (Sysmex corporation, Japan).
2- Serum ferritin level was determined on a chemo luminescence analyzer (chemo luminescence immunoassay; Cobas e411, Roche diagnostics, Switzerland).
3- Transferrin saturation index (TSI) (calculated as serum Fe/TIBC X 100) after serum iron (Fe) and total iron binding capacity (TIBC) determining on chemo luminescence analyzer (Cobas c6000, Roche diagnostics, Switzerland).
4- The concentration of IL6 was measured (after samples were centrifuged and the serum was stored at –80°C) using enzyme linked immunosorbent assay (Bioassay Technology lab, China; catalogue numbers E0090Hu).

Outcomes:

The primary outcomes were to assess levels of IL6 among the studied patients; as well as; determining anemia type according to the patient's iron status.

Statistical analysis:

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 23. The quantitative data were presented as mean, standard deviations and ranges when parametric and median, inter-quartile range (IQR) when data found non-parametric. Also, qualitative variables were presented as number and percentages. The comparison between groups regarding qualitative data was done by using Chi-square test and/or Fisher exact test when the expected count in any cell found less than 5. The comparison between two independent groups with quantitative data and parametric distribution was done by using independent *t*-test while with non-parametric distribution were done by using Mann-Whitney test. The

comparison between two paired groups regarding quantitative data and parametric distribution was done by using Paired *t*-test while with non-parametric distribution was done by using Wilcoxon Rank test. The comparison between more than two groups regarding quantitative data and parametric distribution was done by using One Way ANOVA test while with non-parametric distribution was done by using Kruskall-Wallis test. Spearman correlation coefficients were used to assess the correlation between two quantitative parameters in the same group. A significance level of *p*-value is <0.05.

Results

Regarding demographic data and anthropometric measurements no statistically significant difference between group I+II and group III+IV was found (Table 3). The distribution of brain and solid tumors diagnosis among studied patients is illustrated in Fig. (1).

Concerning the clinical data of the studied group, fatigue being the most common anemia presenting symptom (n=22, 73.3%), with no statistical difference between group I+II and group III+IV regarding blood transfusion need (*p*=0.705) (Table 4).

No statistically significant difference between group I+II and group III+IV regarding 24 hours iron intake with *p*-value=0.149 (Table 5).

Baseline complete blood counts among all groups of the patients showed no statistical difference in hemoglobin levels (*p*=0.091) nor the MCV values, with non-statistical difference in the mean IL6 among the 4 patients' subgroups (*p*=0.561) (Table 6).

No statistically significant correlation found between IL-6 and the other studied parameters (Table 7).

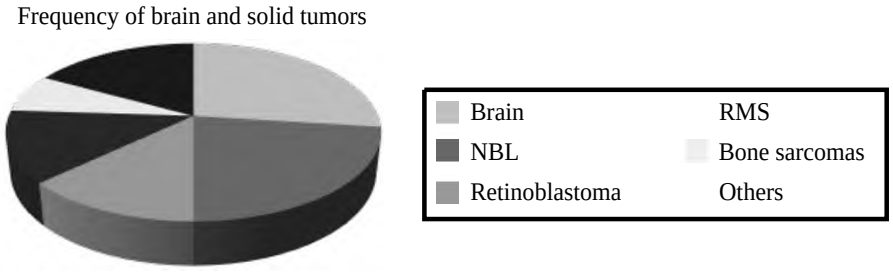

Fig. (1): Frequency of brain and solid tumors diagnoses among studied patients.

NBL : Neuroblastoma. RMS : Rhabdomyosarcoma.
Others: Non-rhabdomyomatous soft tissue sarcoma, germ cell tumor, Wilms tumor.

Table (3): Demographic data and anthropometrics of the studied groups.

	Group I + II No. = 11	Group III + IV No. = 19	Test value	*p*-value
Patients Demographics:				
Age (years):				
Median (IQR)	3 (2 – 9)	6 (4 – 11)	-0.890#	0.374
Range	2 – 13.5	2 – 17		
Gender, n (%):				
Female	4 (36.4%)	8 (42.1%)	0.096*	0.757
Male	7 (63.6%)	11 (57.9%)		
SES, n (%):				
Upper middle	0 (0.0%)	1 (5.3%)	7.162*	0.067
Lower middle	3 (27.3%)	5 (26.3%)		
Upper lower	8 (72.7%)	6 (31.6%)		
Lower	0 (0.0%)	7 (36.8%)		
Consanguinity, n (%):				
No	7 (63.6%)	11 (57.9%)	0.096*	0.757
Yes	4 (36.4%)	8 (42.1%)		
Anthropometric Measurements:				
Wt./age SDS:				
Median (IQR)	-1 (-1 – 1)	0 (-1 – 1)	-0.113#	0.910
Range	-1 – 1	-2 – 2		
Ht/age SDS:				
Median (IQR)	0 (-1 – 1)	1 (0 – 1)	-1.180#	0.238
Range	-1 – 1	-1 – 2		

Table (4): Comparison of the clinical data of the studied groups.

	Group I + II No. = 11	Group III + IV No. = 19	Test value	*p*-value
Symptoms of anemia:				
Fatigue	10 (90.9%)	12 (63.2%)	2.744*	0.098
Palpitation	2 (18.2%)	7 (36.8%)	1.155*	0.282
Pallor	1 (9.1%)	0 (0.0%)	1.787*	0.181
Previous received cancer therapy				
Chemotherapy:				
No	9 (81.8%)	14 (73.7%)	0.258*	0.612
Yes	2 (18.2%)	5 (26.3%)		
Radiotherapy:				
No	11 (100.0%)	18 (94.7%)	0.599*	0.439
Yes	0 (0.0%)	1 (5.3%)		
Duration since last chemotherapy received (months):				
Median (IQR)	4.5 (3 – 6)	2.5 (1 – 3)	–1.609#	0.108
Range	3 – 6	1 – 3		
Surgical treatment:				
No	6 (54.5%)	13 (68.4%)	0.578*	0.447
Yes	5 (45.5%)	6 (31.6%)		
Blood transfusion:				
No	6 (54.5%)	9 (47.4%)	0.144*	0.705
Yes	5 (45.5%)	10 (52.6%)		
Timing of blood transfusion:				
Before chemotherapy	1 (20.0%)	0 (0.0%)	5.250*	0.154
At time of surgery	3 (60.0%)	3 (30.0%)		
At time of presentation	0 (0.0%)	5 (50.0%)		
After chemotherapy	1 (20.0%)	2 (20.0%)		

Table (5): Comparison between group I+II and group III+IV regarding 24 hours iron intake and reported iron profile.

	Group I + II No. = 11	Group III + IV No. = 19	Test value	*p*-value
24 hours iron intake (mg):				
Mean ± SD	6.74±1.21	7.51±1.45	–1.482•	0.149
Range	4.98 – 8.68	4.97 – 9.61		
Transferrin saturation index:				
Median (IQR)	25 (19 − 29)	32 (28 − 37)		
Range	13 – 35	15 – 89		
Ferritin (ng/dl):				
Median (IQR)	40.1 (29.6 − 83.7)	186 (129.8 − 540)		
Range	21.2 – 94	106 – 866		

Table (6): Baseline laboratory characteristics and IL6 levels among the studied groups.

	Group I + II No. = 11	Group III + IV No. = 19	Test value	*p*-value
Hb(g/dl):				
Mean ± SD	9.75±1.06	8.90±1.40	1.752•	0.091
Range	7.5 – 11	4.7 – 10.8		
MCV(fl):				
Median (IQR)	74.67±5.72	75.43±8.57	–0.259•	0.797
Range	69.1 – 88	62 – 102.4		
Retics (%):				
Median (IQR)	0.7 (0.3 – 1.8)	0.8 (0.4 – 1.6)	–0.519#	0.604
Range	0.1 – 3.4	0.2 – 6		
IL6 (Pg/mL):				
Median (IQR)	91.27 (61.36 – 160.1)	68.11 (51.3 – 156.5)	–0.581#	0.561
Range	40.9 – 172.9	28.59 – 326.2		

Table (7): Correlation between IL-6 and hemoglobin and ferritin.

	IL6	
	r	*p*-value
Hemoglobin	0.149	0.431
Ferritin	–0.117	0.539

p-value >0.05: Non significant.
p-value <0.05: Significant.
p-value <0.01: Highly significant.
Spearman correlation coefficient.

Discussion

Regarding the distribution of types of anemia according to iron status among the current included cohort of patients, 20% had absolute iron deficiency, 37% had combined absolute and functional iron deficiency, 16.6% had functional deficiency and 26.4% had anemia not related to iron body status. In several adult studies among solid tumor patients, half of the patients had anemia *[10]*, with CRA patients presenting mainly with functional iron deficiency status related to tumor induced body inflammatory conditions Madeddu et al., *[5]* and Patrick *[11]*.

Among the studied patients, the ferritin level was less than 30ng per dl in 10% and between 30 and 100 in 26.7% more than 100 in 63.3% of patients, in a similar work; *[12]* aimed to evaluate the biochemical features of anemia in cancer patients in an adult group with solid tumors, comparing to the reference value from general population, cancer patients tended to show higher ferritin, tumor necrosis factor alfa (TNFα) and C-reactive protein (CRP) levels, which may explain the debate of cut-off level of ferritin 100 versus 30ug/L among various studies.

Another important marker for inflammation we studied was interleukin 6 (IL6); which during body inflammatory status enhances the production of hepcidin; a key regulator of iron metabolism; we did not find significant difference in IL6 level between patients with absolute and combined iron deficiency on one side and those with functional iron deficiency as regards IL6 level. A possible explanation for the lack of significance of the dependence onto IL6, that the interplay of many inflammatory markers including cytokines, chemokines, leucocytes, prostaglandins, cyclooxygenase, reactive oxygen and nitrogen species occurs in cancer patients; and the dependence on one marker to explain the inflammatory milieu may not be sufficient *[13]*.

Interestingly, an Egyptian study of found that children with acute lymphoblastic leukemia had higher level of hepcidin compared to control group Ragab et al. *[14]*, a group that we excluded for interference of bone marrow infiltrative status with the results for anemia.

The current study showed no significant correlation between hemoglobin level with neither S. ferritin, nor IL6 level. In contrast to our results, Takahashi et al. *[15]* reported hemoglobin levels showing weak negative correlation with IL-6 and hepcidin and a positive correlation with albumin levels used as inflammatory marker rather than nutritional one. Vadhan-Raj *[16]* reported baseline serum levels of IL-6, CRP, and soluble transferrin receptor inversely correlated with hemoglobin levels prior to chemotherapy, supporting their role in anemia of inflammation.

We tried to evaluate RBC parameters in the different anemia subtypes, studying the MCV of the studied patients, there was wide variations in its results, with a mean of 75.15.55 FL and a range of 62-102.4, and there was no difference between the absolute and combined iron deficiency and other groups. Cancer related anemia (CRA) has traditionally been described as a normocytic and normochromic; however, microcytic (MCV <80 fl), hypochromic RBCs are frequently observed *[17]*. Hypochromia frequently develops before microcytosis, a sequence of events that is the reverse of that seen in iron deficiency, with the absolute reticulocyte count not elevated, the white blood cell and the platelet counts are normal or increased, indicating normal granulopoiesis and thrombopoiesis in the face of decreased erythropoiesis *[18]*. In a study of adult patients with cancer, normocytic normochromic anemia was the most frequent type in 67.2% anemic patients when microcytic hypochromic anemia was diagnosed in only 22.9% *[10]*.

As regards the transfusions received in the studied patients, 55% required packed red blood cells transfusion. In agreement with our study Baumeister et al. *[19]* reported patients with head and neck squamous cell carcinoma who required perioperative blood transfusion were 65 (18.4%).

By 24 hours recall, the range of 24 hours iron intake of the current studied patients 4.97-9.61mg per day with 80% (n=24) demonstrating astonishingly an adequate daily iron intake. An Indian study estimated average requirement of iron for normal children ranged from 5.6 to 11.0mg/d in children aged 1-9y *[20]*, whether different recommended daily allowance (RDA) needs to be considered in children with cancer is not clear.

The World Health Organization (WHO) estimates globally that ~273 million young children under 5 years are anemic, among which ~50% are estimated to suffer from iron deficiency *[21]*. In an old study, the nutrient with the lowest intake among children with cancer was iron. Our study showed no statistically significant difference between group

I+II and group III+IV regarding 24 hours iron intake (p=0.149). In 535 child with cancer and its impact on event-free survival (EFS) and overall survival (OS), the study found iron deficiencies in 43.2% of them **[23].**

Based on our results, the study found no statistically significant difference between groups I+II (true and combined iron deficiency) and group III+IV (functional iron deficiency and anemia not related to iron) regarding signs and symptoms of anemia, suggesting that the severity of anemia symptoms might not be related to the type of iron deficiency in cancer patients.

Additionally, there was no statistically significant difference found between the studied groups regarding the percentage of patients who received chemotherapy and radiotherapy, duration since the last chemotherapy received, and history of previous operations. This suggests that the development of anemia in these patients may not be influenced by these treatment modalities or surgical interventions.

Study Limitations:

The small number of patients, the diversity of tumor types and the staging with the involvement of very localized tumors like retinoblastoma compared to very advanced stage as high-risk neuroblastoma in one study group is a major point of defect which may be overcame by focusing on one tumor type and one stage.

Conclusion:

Anemia among cancer patients can be multifactorial with some patients experiencing true iron deficiency anemia, functional or combined true and functional iron deficiency anemia. IL 6 is elevated in cancer patients yet was not reflected on patient's body iron status.

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المسببات المرضية لفقر الدم المرتبط بالسرطان لدى الأطفال والمراهقين المصابين بالأورام الخبيثة

إن التسبب فى فقر الدم لدى مرضى السرطان متعدد العوامل. والتى يمكن أن تُعزى إما إلى تسلل الخلايا السرطانية الى النخاع العظمى مباشرة، وعلاج السرطان بسبب التأثيرات المثبطة للنقي من العلاج الكيميائى إما بمفرده أو بالاشتراك مع العلاج الإشعاعى، وأحيانًا بسبب نقص التغذية. علاج فقر الدم لدى مرضى السرطان، مع استعادة تركيزات الهيموجلوبين الطبيعية؛ يحسن قدرة الدم على حمل الأكسجين ، مما يؤدي إلى تحسين الأعراض المرتبطة بفقر الدم ونوعية حياة المريض. شملت دراستنا الحالية ١٢ أنثى (٤٠,٠٪) و١٨ ذكور (٦٠٪) تراوحت أعمارهم بين ٢ – ١٧ سنة. نقل الهيموجلوبين فى جميع المرضى الذين تتطلب ٥٥٪ منهم نقل خلايا دم حمراء معبأة. أظهرت دراستنا أن نطاق تناول الحديد لمدة ٢٤ ساعة للمرضى الخاضعين للدراسة ٤,٩٧ – ٩,٦١ مع ٨٠ ٪ (ن = ٢٤) يدل على كمية الحديد اليومية الكافية. فيما يتعلق بتعداد الدم الكامل للمرضى الخاضعين للدراسة؛ لم توجد فروق ذات دلالة إحصائية بين المجموعتين المدروسة فيما يتعلق بالمعايير المختبرية.

أظهرت دراستنا الحالية عدم وجود فروق ذات دلالة إحصائية بين المجموعتين الأولى والثانية والمجموعة الثالثة + الرابعة فيما يتعلق بعلامات وأعراض فقر الدم، كما لم يكن هناك فرق معتد به إحصائيًا بين المجموعات المدروسة فيما يتعلق بنسبة المرضى الذين تلقوا العلاج الكيميائي والعلاج الإشعاعى، مع عدم وجود فرق معتد به إحصائيًا بين المجموعتين فيما يتعلق بالمدة منذ آخر علاج كيميائى تلقى وتاريخ العمليات السابقة. أظهرت دراستنا عدم وجود فرق معتد به إحصائيًا بين المجموعتين فيما يتعلق بمستويات الحديد فى الدم والإنترلوكين. أظهرت دراستنا أنه لا توجد علاقة ارتباط ذات دلالة إحصائية بين مستوى الهيموجلوبين وحديد المصل،S. فيريتين، ومستوى إنترلوكين ٦. أظهرت دراستنا الحالية أنه لا توجد علاقة ذات دلالة إحصائية بين مستوى الهيموجلوبين والعلاج الكيميائى، ونقل الدم، وتوقيت نقل الدم فى وقت تقديم فقر الدم وعملية التاريخ.