Levels of Tumor Necrosis Factor Alfa and Iron Status in Pediatric Patients with Newly Diagnosed Solid Tumors

By

Sara Mostafa Makkeyah [1], Mohamed Abd El-Mohsen El-Laboudy [1], Menna Allah Zakaria Mohammad Ali Abou Elwafa[2], Sara Sobhy Mohamed Selim[1], Salwa Mostafa Abd El Kader [1]

[1]Pediatric Hematology/Oncology, Childrens Hospital, Ain Shams University, Cairo, Egypt.

[2] Clinical Pathology, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Corresponding author: Sara Mostafa Makkeyah

Email: smakkeyah@med.asu.edu.eg **Mobile**: 02 01140105222

ABSTRACT

Background: *Cancer-related anemia (CRA) occurs in a significant proportion of cancer patients and affects disease progression, treatment efficacy, and survival. The chronic inflammatory state associated with cancer is believed to be a major cause of CRA. Tumor necrosis factor alfa (TNF-α) plays a crucial role in the pathogenesis of cancer-related anemia (CRA).*

Aim: *To evaluate TNF-α level, erythropoietin (EPO), and iron status in children with newly diagnosed solid tumors.*

Methods: *This cross-sectional study included 42 children with newly diagnosed solid tumors, recruited from the Pediatric Oncology Clinic at Ain Shams University Children's Hospital by random sampling during the period from June 2022 to February 2023. Twenty-five age- and sex-matched children were enrolled as hospital-based controls. All study participants were subjected to full history taking, through clinical examination and assessment of CBC, iron profile, serum TNF-α and EPO levels.*

Results: *Out of 42 cancer patients, 48%(n=20) were non-anemic (group 1) and, 52%(n=22) were anemic (group 2) with 54.5% (n=12) having functional iron deficiency (FID) anemia. In both patient groups, significantly higher TNFα and lower EPO levels were found compared to controls. Among the anemic group, TNFα negatively correlated with both EPO level and reticulocyte count (p= 0.024) and (p= 0.211) respectively.*

Conclusion: Patients with CRA have evident state of FID anemia, elevated TNFα and reduced EPO levels.

Keywords: Pediatric solid tumors, Anemia, TNF-α, EPO, FID, CRA

Introduction

Anemia represents the most encountered hematologic abnormality affecting more than half of the children below the age of 5 years **(Chaparro & Suchdev 2019).** Anemia among children is defined by a hemoglobin or a hematocrit level lower than two standard deviation (SD) below the mean for age and sex for the normal population **(WHO, 1997).**

Cancer-related anemia (CRA) occurrs in more than 30% of cancer patients at initial disease presentation affecting survival, disease progression, treatment efficacy, for which early detection and appropriate management is mandatory **(Zhang et al., 2017).** CRA occur independently from concurrent antineoplastic therapy, typically because of the chronic inflammatory state associated with the cancer disease **(Madeddu et al., 2018),** which leads to shortened erythrocyte survival, bone marrow (BM) hypo-proliferation, impaired erythropoietin (EPO) production, as well as; altered body iron metabolism (**Adamson 2008**).

Historically, proinflammatory cytokines (e.g., TNF-a, IL-1, IL-6), released by the cancer and activated immune cells in response to malignancy, have been identified to result in CRA **(Means & R. T. 1995),** through maintaining a state of chronic body inflammation that's associated with increased production of reactive oxygen species (ROS), which acts to inhibit EPO synthesis (**Macciò et al., 2015**).

Adult studies proved that variable cancer related factors affect the severity of the CRA presentation, including the type of underlying malignancy and disease stage at time of diagnosis (**Ludwig, et al. 2004**).

Little is known and reported about CRA etiopathogenesis among pediatric solid tumor cancer patients; for which we considered it crucial to establish evidence about inflammatory marker TNF-α and its relation to ferritin as well as EPO among solid tumor pediatric patients.

Ethical consideration

(1) An approval of the Ethical Committee of Faculty of Medicine, Ain Shams University under acceptance number FMASU MS 384/ 2022 was obtained.

(2) An Informed consent was taken from parents or care givers before getting involved in study.

(3) Financial disclosure: The author received no financial support for the research.

(4) The authors declared no potential conflicts of interest with respect to the research and publication of the research.

(5) The data of the study are confidential, and the patient has the right to keep it.

(6) The patient has the right to refuse participation in the study.

Sample size

By using G power program for sample size calculation, setting power at 80%, alpha error at 5% and assuming medium effect size difference (0.03) in tumor necrosis factor alpha (TNF-α) level between patients with cancer-related anemia, patients with cancer without anemia, and healthy controls; based on that, a sample size of at least 20 patients with cancer-related anemia and 20 patients with cancer without anemia and 20 healthy control subjects be sufficient to achieve study objective.

Subjects and methods

Study design and population

This cross-sectional study was conducted on 42 children with newly diagnosed brain or solid tumors, recruited from the Pediatric Oncology Clinic at Ain Shams University Children's Hospital by random sampling during the period from June 2022 to February 2023. Twenty-five age- and sex-matched children were enrolled as hospital-based controls.

Inclusion criteria:

- Gender: Both males and females included.
- Age: 2-18 years.
- Patients with CNS tumors and other solid tumors at time of initial presentation and before the start of any cancer therapy.

Exclusion Criteria:

- Patients with hematologic malignancies.
- Patients with vascular tumors or tumors involving gastrointestinal system.
- Relapsed/refractory cancer patients.

Study groups

I. Patient groups

Patients were subdivided into two groups according to the WHO definition and grading of anemia according to age and gender (**Rambod et al.,2008**):

- Group 1 (n=20) for patients without CRA
- Group 2 (n=22) included those with CRA

Based upon iron status, group 2 patients were classified into absolute iron deficiency (AID, serum ferritin <30ng/mL & transferrin saturation (TSI) <20%), functional iron deficiency (FID, serum ferritin >100ng/mL & TSI 20-50%) or combined iron deficiency (CID, serum ferritin 30-100ng/mL & TSI <20%). Disease status at initial presentation was classified according to the National cancer institute (NCI) tumor staging criteria into; locally confined, locally advanced, or metastatic (**Jeffrey et al., 2008**).

II. Control group

Controls were recruited from the outpatient clinic, by random sampling. They were subjected to full history taking, clinical examination and measurement of serum TNF α and EPO levels.

Methods

All the studied groups were subjected to:

I. Detailed history taking

Detailed medical history was taken from all patients regarding demographics including age and gender, socio economic status according to the Egyptian based score by ***El-Gilany et al 2012***, tumor pathological type, site of cancer, stage, history of previous blood loss, treatment modalities, chemotherapy, Hb drop during treatment, and the need for red cell transfusion.

II. Clinical examination

All patients were subjected to thorough clinical examination with recording of the following: anthropometric measurements (weight, height, body mass index), signs of anemia (pallor, hair and skin integrity, full examination of the pulse), any bleeding signs, or signs related to previous surgical intervention, detailed systemic examination including cardiac examination, neurological examination, as well as abdominal examination.

III. Laboratory assessment

Blood samples were collected from the patients before the start of chemotherapy or blood transfusion. The following tests were performed:

- Complete blood counts (CBC) (Sysmex XN-1000, japan) and reticulocyte count.
- Iron profile (serum iron, total iron binding capacity (TIBC), and serum ferritin (Cobas c6000 & e411, Roche diagnostics, Switzerland).
- Results of Bone marrow biopsies were reviewed at time of diagnosis.
- TNFα level was measured in serum samples using enzyme linked immunosorbent assay ELISA (Bioassay Technology lab, China; catalogue numbers E0082Hu).
- EPO level was measured in serum samples using ELISA (Bioassay Technology lab, China; catalogue numbers E1029Hu).

Statistical Analysis

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 23. The comparison between groups regarding qualitative data was done by using Chi-square test and/or Fisher exact test. 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. Spearman correlation coefficients were used to assess the correlation between two quantitative parameters in the same group.

Results

The study results are presented in the following tables and figures.

Table (1) Demographic data of the studied groups

		Control N = 25	**Group 1 N = 20**	**Group 2 N = 22**	**P-value**
Age (yr) Median (IQR)		7 (4.5 – 12)	9.5 (3 – 12.5)	6 (2.9 – 9)	0.471
Gender N (%)	**Female**	15 (60.0)	9 (45.0)	10 (45.5)	0.505
	Male	10 (40.0)	11 (55.0)	12 (54.5)	
Nationality N (%)	**Non-Egyptian**	0 (0.0)	2 (10.0)	4 (18.2)	0.091
	Egyptian	25 (100.0)	18 (90.0)	18 (81.8)	
Socioeconomic score N (%)	**Low**	17 (68.0)	11 (55.0)	12 (54.5)	0.793
	Very low	4 (16.0)	4 (20.0)	3 (13.6)	
	Middle	3 (12.0)	5 (25.0)	6 (27.3)	
	High	1 (4.0)	0 (0.0)	1 (4.5)	
Consanguinity N (%)	**No**	22 (88.0)	12 (60.0)	15 (68.2)	0.089
	Yes	3 (12.0)	8 (40.0)	7 (31.8)	

Regarding demographic data no significant difference was found between patients and controls.

Table (2) Comparison of the clinical data of the patient groups

	Group 1 N = 20	**Group 2 N = 22**	**P-value**
Symptoms N (%)			
Palpitation	3 (15.0%)	12 (54.5%)	**0.002**
Pallor	0 (0.0%)	9 (40.9%)	**0.005**
Fatigue	7 (35.0%)	14 (63.6%)	0.095
Transfusion in the past 3 months	0 (0.0%)	3 (13.6%)	**0.04**
Diagnosis N (%)			
Brain tumor	8 (40.0%)	6 (27.3%)	0.38
NBL	1 (5.0%)	6 (27.3%)	**<0.001**
NRSTS	3 (15.0%)	4 (18.2%)	0.78
RMS	2 (10.0%)	1 (4.5%)	0.49
Bone sarcoma	3 (15.0%)	1 (4.5%)	0.24
Others (miscellaneous)	3 (15.0%)	4 (18.2%)	
Staging N (%)			
Metastatic	2 (10.0%)	12 (54.5%)	**0.008**
Locally advanced	9 (45.0%)	6 (27.3%)	
Locally confined	9 (45.0%)	4 (18.2%)	

NBL: neuroblastoma; RMS: rhabdomyosarcoma; NRSTS: non-rhabdomyomatous soft tissue sarcoma

This table shows that group 2 patients had a higher frequency of anemic symptoms at presentation and blood transfusion in the last 3 months. They also had a significantly higher frequency of advanced (metastatic and local) disease status than group 1.

Table (3) Baseline laboratory characteristics among patient groups

	Parameter	Group 1 N =20	Group 2 N = 22	P-value
CBC	Hb (g/dl) Mean ± SD	12.39 ± 0.79	9.57 ± 1.30	**<0.001**
	MCV (fl) Mean ± SD	77.48 ± 3.57	77.39 ± 6.80	0.956
	MCH (pg) Mean ± SD	25.45 ± 1.92	24.69 ± 2.85	0.322
	Retics (%) Median (IQR)	0.8 (0.5 – 1.5)	0.8 (0.6 – 1.8)	0.477
BMA	Not done N (%) Infiltrated N (%) Not infiltrated N (%)	15 (75.0%) 0 (0.0%) 5 (25.0%)	16 (72.7%) 5 (22.7%) 1 (4.5%)	**0.022**
Iron profile	S. iron (ug/dl) Median (IQR)	76.5 (54 – 102)	68.5 (48 – 95)	0.442
	S. ferritin (ng/ml) Median (IQR)	118.1 (64.91 – 554)	104.55 (33.4 – 343.9)	0.302
	TIBC (ug/dl) Mean ± SD	314.55 ± 59.65	297.32 ± 53.72	0.330
	TSI (%) Median (IQR)	23.7 (15.85 – 34.8)	25.25 (17.2 – 30.8)	0.772

Baseline complete blood counts among group 2 showed a significantly lower hemoglobin level than group 1 patients. Interestingly, the reticulocyte count showed no statistical difference between group 1 and 2 patients (Table 3). Bone marrow biopsy was performed in 25% of patients with significantly higher prevalence of BM infiltration among group 2 patients (p= 0.022).

Iron profile showed no difference between group 1 and 2, with notably elevated ferritin >100ng/mL in both groups (p=0.302) (table3). Twelve out of 22 (54.5%) anemic patients fulfilled the diagnostic criteria of FIDA, while 36.4% were classified laboratory as CIDA.

Table (4) EPO and TNFα among the study groups

	Control N = 25	**Group 1 N = 20**	**Group 2 N = 22**	**P-value**
EPO (mIU/ml) Median (IQR)	357 (248.1 – 513)	86.98 (61.5 – 112.5)	92.98 (69.2 – 115.2)	**<0.001**
TNFα (ng/L) Median (IQR)	79.3 (63.3 – 98.3)	353.2 (301.9 – 457.9)	306.2 (253.5 – 491.1)	**<0.001**

Post hoc analysis

	Control Vs group 1	Control Vs group 2	Group 1 vs group 2
EPO	**<0.001**	**<0.001**	0.481
TNF-α	**<0.001**	**<0.001**	0.465

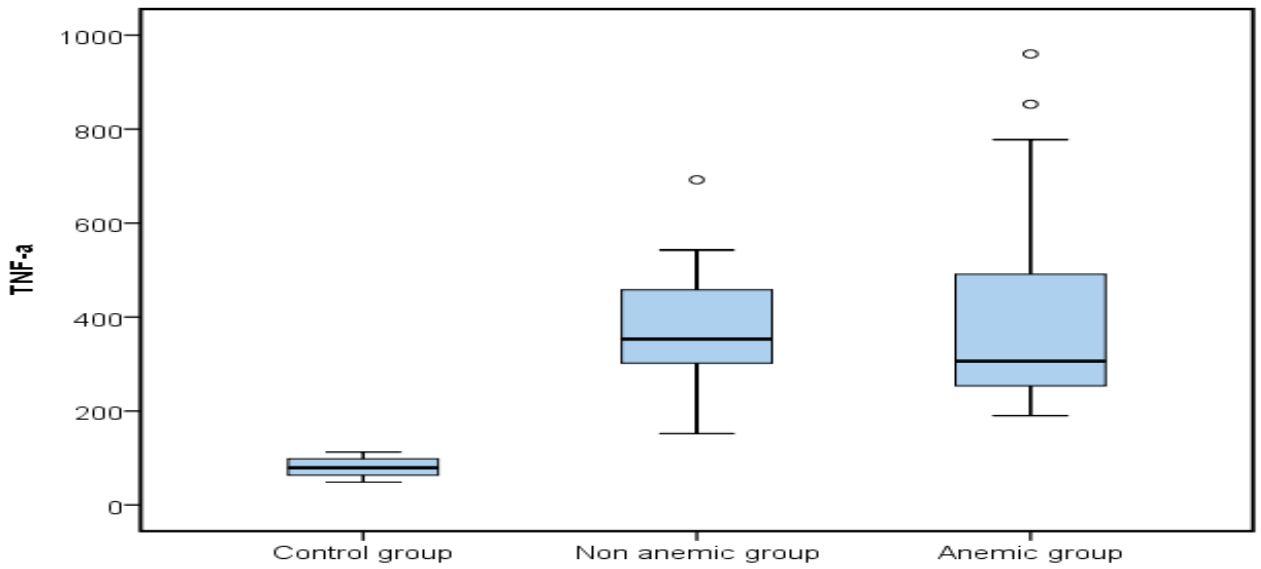


Figure (1) Comparison of TNFα levels among study groups

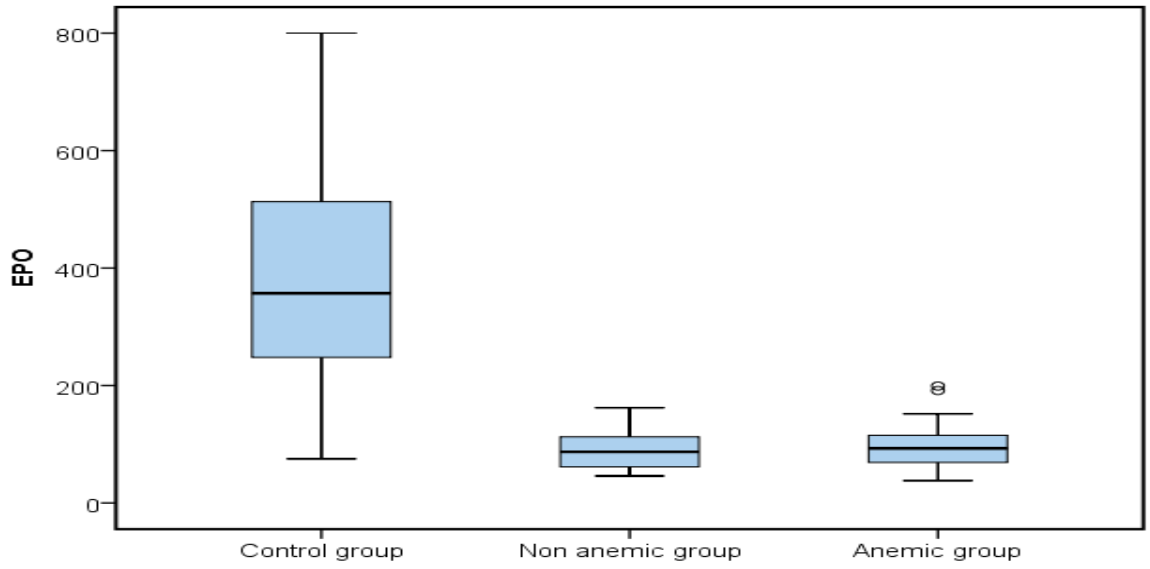
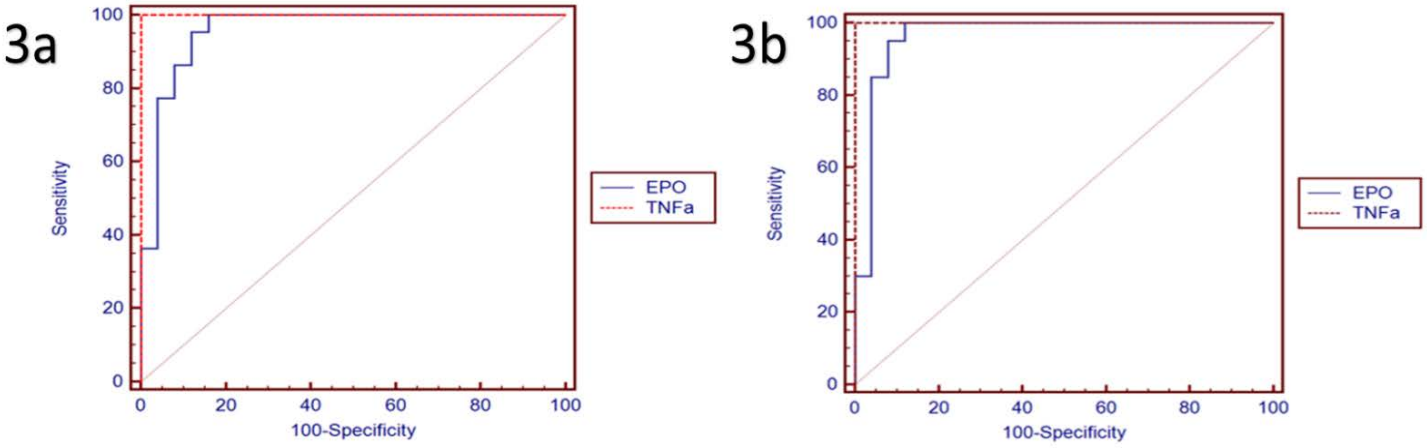


Figure (2) Comparison of EPO levels among study groups

Table 4 and figures 1 & 2 show a statistically significant higher TNFα, and lower EPO levels among group 1 and 2 compared to control, yet post hoc analysis confirmed no significant difference in neither TNFα nor EPO responses between group 1 and 2 patients.

Among group 2, TNFα showed negative correlation with both EPO level and reticulocyte count (r=-0.481 and p=0.024) and (r=-0.084 and p=0.211) respectively which was not evident among group 1. TNF-α was significantly lower among anemic group with locally confined rather than advanced and metastatic disease (P= 0.046).

Figure (3a) Receiver operating characteristic curve (ROC) for EPO and TNFα levels to differentiate



between control group and group 2, (3b) ROC curve for EPO and TNFα levels to differentiate between controls and group 1.

The ROC analysis showed 100% sensitivity and 80-84% specificity of both EPO and TNFα in differentiating control from patients' groups at a cut off EPO ≤162.2 and ≤199.2 mIU/ml in group 1 and 2, and TNFα cut off > 112.7 ng/Lin both groups (Figure 3a & 3b).

Discussion

Anemia among pediatric population represents a group of heterogenous blood disorders of varying acquired and inherited causes, with CRA being a separate entity with pathogenesis resembling anemia of chronic inflammatory conditions (**Madeddu et al., 2018** and **Patrick 2022**).

In the current study among the enrolled patients' group with CRA, 86% of them gave history of anemic symptoms starting 2-4 months prior to their cancer diagnosis, with 13% of them requiring PRBCs transfusion. Similarly, reported **by Ludwig and Strasser 2001** that 30% of cancer patients presents with variable CRA related symptoms and signs including fatigue, lethargy, and dyspnea that can compromise performance status, as well as impairing therapeutic responses to chemotherapy and radiotherapy.

As reported by one of the largest international surveys conducted over 10 different European pediatric oncology treating centers, CRA diagnosis was encountered initially among 80% of the enrolled patients, with bone sarcoma, NBL and brain tumors being the main diagnoses presenting with variable degrees of CRA requiring transfusion in 40-70% of the patients (**Michon 2002**),

Interestingly observed in the current study, the most frequent solid tumors diagnoses among the enrolled patients were brain tumors, sarcomas followed by NBL, with more than 80% of the NBL patients' presenting initially with CRA, with a strikingly observed higher percentages of advanced/metastatic disease presentation among solid tumor patients presenting with CRA. In the opposite ends of the disease spectrum; reports in adult cancer patients, CRA prevalence, like in pediatric data, showed variation among cancer types, with the highest percentage reported in pulmonary and gastrointestinal carcinomas (**Schwartz & R. 2007**).

In a single center report, AID was encountered in 63% of treated cancer patients, for which authors were considering a baseline testing of iron profile (TSI and ferritin) a standard practice in CRA patients at initial presentation (**Gilreath et al., 2014**) who would clearly benefit from iron replacement therapy, unlike CRA patients with a state of FID.

Among the current study with CRA, peripheral blood counts showed variation in the anemia morphologic type between micro and normocytic types, yet with evidently low median reticulocyte count reflecting improper marrow regeneration, Expectedly in both patients' groups, iron profile at baseline presentation showed evidence of impaired body iron metabolism with elevated median serum ferritin >100 ng/ml, as well as, among CRA patients >50% expressed evident FIDA state.

A previous report by Adamson, 2008 described CRA to be often normocytic normochromic (**Adamson 2008**), with **Rodgers et al., 2017** confirming CRA one of the hypo-proliferative anemias presenting reticulocytopenia and low reticulocyte index and a concomitant state of FID. Moreover, excessively produced TNFα among patients with cancer, has shown to induce apoptosis of immature erythroblasts, and to reduce the responsiveness of erythroid progenitors to EPO (**Buck et al., 2009**).

In the current study, highly significant higher TNFα and lower EPO levels among cancer patients was found, with unexpectedly low median EPO levels among CRA group comparable to patients without CRA. It has also been shown among CRA patients' group a negative correlation between TNFα levels and both EPO level and reticulocyte count which was not evident in the non-anemia patients representing a state of BM hypo-regeneration among CRA patients.

Though rarely reported, in a similar work by the Chinese group of Qingdao, exploring EPO and TNFα levels among pediatric cohort with CRA, interestingly reported higher levels of TNFα among CRA patients which was negatively correlated with the patient's hemoglobin levels, yet EPO among CRA patients showed proportionate high levels, with the authors concluding TNFα role in CRA by a non-EPO related etiology (**Hao et al., 2020**)

Conclusion

Pediatric solid tumors show a varying incidence of CRA development at initial disease presentation with advanced/metastatic diseases and neuroblastoma being the most frequently involved. Patients with CRA tend to have evident state of reticulocytopenia, as well as associated FID anemia, elevated TNFα and reduced EPO levels.

Recommendations

Larger scales of pediatric baseline surveillance for CRA among solid tumor patients, especially those presenting at advanced stages of the disease with the possible role of TNFα as a marker of chronic inflammation and BM hypo regeneration.

Limitations

The current study was conducted on a limited number of patients, which mandates recommending further studies on larger patient population. In addition to the diversity in patients' diagnosis, which may affect generalization of results, thus recommending the focus of future studies on certain stages and diagnosis of cancer.

References

1. **Adamson J. W. (2008).** The anemia of inflammation/malignancy: mechanisms and management. Hematol. Am. Soc. Hematol. Educ. Program 2008, 159–165.doi: 10.1182/asheducation-2008.1.159

2. **Buck, I., Morceau, F., Grigorakaki, C., et al., (2009).** Linking anemia to inflammation and cancer: the crucial role of TNF alpha. Biochem. Pharmacol. 77, 1572–1579. doi: 10.1016/j.bcp.2008.12.018

3. **Chaparro CM, Suchdev PS. (2019):** Anemia epidemiology, pathophysiology, and etiology in low- and middle-income countries. Ann NY Acad Sci. 2019;1450(1):15-31.

4. **El-Gilany, A., El-Wehady, A., and El-Wasify, M. (2012).** Updating and validation of the socioeconomic status scale for health research in Egypt. Eastern Mediterranean health journal, 18(9).

5. **Hao, Wufeng Xu, Min Wei1, et al., (2020):** Correlation between Erythropoietin and Tumor Necrosis Factor Alpha in Children with Malignant Tumor Anemia. Advances in Clinical Medicine, 10(4)

6. **Jeffrey A. Gilreath, David D. Stenehjem, and George M. Rodgers (2014):** Diagnosis and treatment of cancer-related anemia American Journal of Hematology, Vol. 89, No. 2,

7. **Ludwig, H., and Strasser, K. (2001).** Symptomatology of anemia. Semin. Oncol. 28(2 Suppl. 8), 7–14. doi: 10.1016/S0093-7754(01)90206-4

8. **Ludwig H, Van Belle S, Barrett-Lee P, et al. (2004):** The European Cancer Anaemia Survey (ECAS): a large, multinational, prospective survey defining the prevalence, incidence, and treatment of anaemia in cancer patients. Eur J Cancer; 40:2293–306.

9. **Macciò, A., Madeddu, C., Gramignano, et al. (2015).** The role of inflammation, iron, and nutritional status in cancer-related anemia: results of a large, prospective, observational study. Haematologica 100, 124–132. doi: 10.3324/haematol.2014.112813

10. **Madeddu, C., Gramignano, G., Astara, G., et al., (2018):** Pathogenesis and treatment options of cancer related anemia: perspective for a targeted mechanism-based approach. Frontiers in physiology, 9, 1294.

11. **Means, R. T. (1995).** Pathogenesis of the anemia of chronic disease: a cytokine mediated anemia. Stem Cells 13, 32–37. doi: 10.1002/stem.5530130105

12. **Michon J. (2002):** Incidence of anemia in pediatric cancer patients in Europe: results of a large, international survey. Med Pediatr Oncol; 39:448–50.

13. **Patrick G. Gallagher. (2022):** Anemia in the pediatric patient. Blood, VOLUME 140, NUMBER 6 571-593

14. **Rodgers, G. M. III, Gilreath, J. A., et al. (2017).** NCCN. Cancer- and Chemotherapy-induced Induced Anemia, Version 2.2017.

15. **Schwartz, R. N. (2007).** Anemia in patients with cancer: incidence, causes, impact, management, and use of treatment guidelines and protocols. Am. J. Health Syst. Pharm. 64(3 Suppl. 2), S5–S13. doi: 10.2146/ajhp 060601

16. **World Health Organization; (1997):** Iron Deficiency: Indicators for Assessment and Strategies for Prevention. Geneva, Switzerland: World Health Organization. WHO/NUT

17. **Zhang, L. L., Zhou, et al. (2017).** Combined prognostic value of pretreatment anemia and cervical node necrosis in patients with nasopharyngeal carcinoma receiving intensity modulated radiotherapy: a large-scale retrospective study. Cancer Med. 6, 2822–2831. doi: 10.1002/cam4.1233