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Assessment of serum soluble transferrin receptor in adult Egyptian aplastic anemia patients

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Abstract

Aplastic Anemia (AA) is one of the life-threatening bone marrow failure syndromes. One of the main pathologies of AA is reduced erythropoietic activity evidenced by decreased soluble transferrin receptor (sTfR) levels which results in minimal iron utilization and accumulation of iron in tissues in the form of ferritin. This study aimed to measure serum level of sTfR in adult AA patients and correlate it with the severity of the disease and the response to treatment. The study included 35 randomly selected AA patients recruited from the Hematology Department, aged from 17 to 66 years and 27 normal controls of matched age and sex. The level of sTfR was measured by using an enzyme linked immunoassay. The median level of sTfR was significantly lower in AA cases than in controls (17.9 nmol/l, ranged 9.87–30.42 nmol/l vs. 52.2 nmol/l; ranged 29.74–86.84 nmol/l, (p<0.001). The concentration of sTfR was significantly lower in the Very Severe Aplastic Anemia (VSAA) patients in comparison to Severe Aplastic Anemia (SAA) 34.27 nmol/l (ranged 31.05 – 45.41 nmol/l) vs. 55.15 nmol/l (ranged 48.78–63.88 nmol/l), respectively, p= 0.004). The level of sTfR in responders to immunosuppressive treatment did not show any difference in comparison to non-responders [55.15 nmol/l (ranged 47.36 – 65.35 nmol/l) vs. 48.26 nmol/l (ranged 34.62 – 60.39 nmol/l), (p=0.808). In conclusion, sTfR level was significantly lower in AA cases than controls. The sTfR concentration expresses the erythropoietic activity in AA patients and can be an indicator of severity of bone marrow failure.

Keywords: soluble transferrin receptor, Severe Aplastic Anemia, erythropoiesis.

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Introduction

Aplastic anemia (AA) is a bone marrow disease with heterogeneous etiological background which mainly caused by apoptosis/cell death of hematopoietic progenitors. The defining features are pancytopenia, reticulocytopenia, hypocellularity of bone marrow and low of hematopoietic cells.[1]

AA occurs due to failure of the bone marrow precursor stem cells to produce adequate amount of mature hematopoietic cells. It is categorized in terms of severity from mild to moderate pancytopenia to complete hematological collapse. The modified Camitta criteria classify aplastic anemia into three categories on the basis of bone marrow

cellularity, absolute neutrophil count, platelet count, and reticulocyte count.[2, 3]

Erythropoietic activity is highly affected by iron regulation as they are both intrinsically regulated.[7] Dysregulation of iron balance is well documented in beta thalassemia in which ineffective erythropoiesis is the main cause. On the other hand, iron stores and the overall iron status are poorly elucidated in bone marrow failure where erythropoiesis is minimal.[4]

Soluble transferrin receptors (sTfRs) are proteins found in blood, cleaved from the membrane-bound transferrin receptors found on nearly all cells.[4] Soluble TfR is a truncated monomer of tissue receptor, lacking its first 100 amino acids, which circulates in the form of complex transferrin and its receptor.[5]

sTfR serum concentration is correlated to tissue receptor expression, accordingly, provides a clinical measure of the potential for cell proliferation (erythropoiesis). The levels of sTfR are decreased in conditions with decreased erythropoietic activity such as cancer chemotherapy. On the other hand, they are increased when erythropoiesis is stimulated by hemolysis or ineffective erythropoiesis.[6]

Measurement of sTfR is very useful to investigate the pathophysiology of anemia, quantitatively evaluating the absolute rate of erythropoiesis and the adequacy of marrow proliferative capacity for any given degree of anemia. In terms of its significance from a prognostic perspective, it reflects the erythropoietic response to various forms of therapy, in particular allowing to predict the response when changes in hemoglobin are not yet apparent.[7]

Low erythropoietic activity in AA as evidenced by reduced sTfR levels, leads to minimal iron utilization and accumulation of iron in the tissues as ferritin. This process results in precipitation of iron in the form of ferritin which stimulates the production of hepcidin and dysregulation of iron homeostasis. Increased marrow iron leads to limitation in intrinsic hematopoiesis by increasing in oxidative stress. Therefore, better understanding of iron regulation in AA is essential for effective therapeutic options. Accordingly, the aim of this study was to assess the level of serum sTfR in adult aplastic anemia patients and its correlation with the severity of the disease and the response to treatment.

Subjects and Methods

Patients' selection

This was a case control study that included 35 randomly selected aplastic anemia patients recruited from inpatient and outpatient clinics of the Hematology Department, Ain Shams University hospital during the period between March 2023 and March 2024. They aged from 17 to 66 years, in addition to 27 normal controls of matched age and sex.

The study patients were diagnosed in Accordance with the International Agranulocytosis and Aplastic anemia study (1987), then further subdivided according to the Camitta criteria into mild, moderate and severe groups.[8]

The criteria of AA severity included absolute neutrophil count (ANC) <0.5x10^9/l or platelet count (PLT) <20~10/l or hemoglobin (HGB) <80 g/l, absolute value of reticulocytes <20~10/l before treatment.[2,3]

Patients with inherited syndromes of bone marrow failure, secondary infiltrative bone marrow entities, patients presenting with secondary aplastic anemia to radiotherapy or chemotherapy and hypoplastic Myelo-Dysplastic Syndrome (MDS) were excluded from the study.

All patients were subjected to careful history taking with emphasis on history of drugs, radiation and toxic chemicals, as well as to clinical examination.

Laboratory data for study patients were collected from patients' files at the hospital. These mainly included complete blood count (CBC), renal and hepatic function tests, autoimmune markers, viral markers for Hepatitis C virus, and Ferritin level.

All patients also underwent a bone marrow aspiration and biopsy as part of the aplastic anemia workup.

The enzyme linked immunoassay (ELISA) was performed for quantitative estimation of Serum of soluble transferrin receptor level (sTfR). We used commercial ELISA kits (Catalog number E0281Hu, Biotechnology ELISA Kit, Zhejiang, China), according to the manufacturer's instructions. From each study subject a venous blood sample (2 ml) was collected, centrifuged at 2000 g for 10 minutes, and serum samples were collected and used in the ELISA.

For quantitative measurement of sTfR levels in serum a log/log curve of absorbance values of kits standards was drawn. The Kit sensitivity for sTfR was 0.85 nmol/l. For any results above the test linearity (1000 nmol /l), the sera were diluted and the obtained results multiplied with the dilution factor.

Treatment of aplastic anemia

Study patients were offered different options of treatment, including immunosuppressive therapy in the form of cyclosporine and anti-thymocyte globulin (ATG) for 6 months or supportive treatment in the form of blood products transfusion. For the response to treatment patients were divided according to the guidelines for the diagnosis and management of adult aplastic anemia[9] into:

1- Complete therapeutic response (CR): HGB>100g/L, PLT> 100×10/l and ANC>1.5×10^9/l

2- No treatment response (NR): The patient is not weaned from blood product transfusion supportive care and (or) hematology tests still meet Severe Aplastic Anemia (SAA) standards.

Regarding the controls, the pool recruited did not suffer any cytopenia or other diseases.

Statistical Analysis

The data were analyzed employing Microsoft Excel 2010 (Microsoft Corporation, Seattle, WA, USA) and the Statistical Package for Social Sciences (SPSS) version 23 (IBBM. Chicago, IL, USA). The quantitative variables for parametric data are presented as mean, standard deviations and ranges and for non-parametric data as median and interquartile range. Qualitative variables are presented as numbers or percentages. The comparison between groups with qualitative data was done using the Chi-square test. The unpaired t-test was done for normally distributed continuous variables. The Mann–Whitney test was applied for the non-normally distributed continuous variables. Spearman correlation was used to assess the correlation between two quantitative parameters of the same group. A p value <0.05 was considered significant.

Results

This study included 35 aplastic anemia cases divided into 54% males (n=19) and 46% females (n= 16) and 27 control individuals divided into 55% males (n=15) and 45% females (n=12). The mean age of the AA cases was 35.63 ± 15.23 years ranged from 17 to 66 years vs. 34.33 ± 15.08 years, ranged from 17 to 58 years in the controls. There was no age and gender difference between patients and controls (p>0.333 and p>0.010, respectively).

The aplastic anemia patients were divided based on the severity of disease, according to the International Agranulocytosis criteria into two groups: very severe, included 80% of patients (n=28) and 20 % severe (n= 7). About 91.4 % of aplastic anemia patients (n=32) received immunosuppressive therapy in the form of cyclosporine and Anti-thymocyte globulin (ATG) for 6 months and the remaining 8.6% (n=3) were on supportive transfusion upon demand.

Treatment response

The patients were offered different options of treatment, including immunosuppressive therapy in the form of cyclosporine and ATG for 6 months or supportive treatment in the form of blood products transfusion. The response to immunosuppressive therapy was detected in 15 patients (42.9%) after 6 months of follow-up.

Different types of infections (e.g.: bacterial, viral or fungal) occurred in 60% of cases (n=21). The mortality rate among our patients was 65.7% of the patients (n= 23) mainly due to life threatening infections (bacterial or fungal infection (Table 1).

Table 1. Description of the parameters studied among the 35 study patients.

Studied parameters		Parameters data
Age	Mean ± SD	35.63 ± 15.23 years
	Range	17- 66 years
Sex	Female	16 (45.7%)
	Male	19 (54.3%)
TLC	Median (IQR)	1.7 (0.7 – 2)
	Range	0.1 – 7.6
HBG	Mean ± SD	7.74 ± 1.22
	Range	5.9 – 11
PLT	Median (IQR)	16 (9 – 26)
	Range	1 – 277
Kidney function tests	Normal	35 (100.0%)
	Impairment	0 (0.0%)
Liver function tests	Normal	31 (88.6%)
	Impairment	4 (11.4%)
Immunosuppressive	Negative	3 (8.6 %)
	Positive	32 (91.4%)
Mortality	Alive	12 (34.3%)
	Died	23 (65.7%)
Viral markers	Negative	34 (97.1%)
	HCV	1 (2.9%)
Ferritin	Median (IQR)	2613.5 (1573.5 – 5865)
	Range	1400 – 6630
Severity of Aplastic Anemia	Very Severe	28 (80.0%)
	Severe	7 (20.0%)
Response to treatment	No	20 (57.1%)
	Yes	15 (42.9%)
Bacterial, viral, or fungal infection	No	14 (40.0%)
	Yes	21 (60.0%)

TLC: Total leukocytic count, HB: hemoglobin, PLT: Platelet counts, IQR: Interquartile range.

The median level of sTfR was significantly lower in AA cases than controls (17.9 nmol/l, range 9.87 – 30.42 nmol/l vs. 52.2 nmol/l; range 29.74 – 86.84 nmol/l, p<0.001) (Table 2).

No significant correlation was observed between sTfR (nmol/l) and other studied parameters (Table 3).

Table 2. Comparison of serum soluble transferrin receptor factor (sTfR) between the cases and controls.

Transferrin receptor	Patients group No. = 35	Control group No. = 27	p-value
Median (IQR), nmol/L	17.9 (12.4 – 22.5)	52.2 (38.0 – 62.5)	<0.001
Range, nmol/L	9.87 – 30.42	29.74 – 86.84	

IQR: Interquartile range. $p \leq 0.05$ is significant.

Table 3. Correlation of transferring receptor factors (sTfR) with the other parameters studied.

Studied parameters	Transferrin receptor	
	r	p-value
Age	-0.318	NS
TLC	-0.120	NS
HGB	0.286	NS
PLT	-0.014	NS
Ferritin	-0.130	NS

TLC: Total leukocytic count, HB: hemoglobin, PLT: Platelet counts. $p > 0.05$ is not significant (NS).

Serum concentration of sTfR was significantly lower in the Very Severe Aplastic Anemia (VSAA) patients in comparison to SAA; 34.27 nmol/l (31.05 – 45.41 nmol/l) vs. 55.15 nmol/l (48.78 – 63.88 nmol/l), respectively, (p= 0.004).

There was no difference between the levels of sTfR in responders to immunosuppressive treatment and the non-responders [55.15 nmol/l (47.36 – 65.35 nmol/l) vs. 48.26 nmol/l (34.62 –60.39 nmol/l)] (p=0.808). There was no statistically significant correlation between levels of sTfR (nmol/l) and clinical manifestations of studied patients. (Table 4)

Table 4. Correlation of sTfR with the other parameters studied among the patients group.

		Transferrin receptor		p-value
		Median (IQR)	Range	
Sex	Female	55.15 (49.93 - 61.95)	34.02 - 86.84	NS
	Male	48.26 (36.32 - 65.27)	29.74 - 78.63	
Liver function	Normal	51.96 (38 - 61.4)	31.05 - 86.84	NS
	Impairment	58.33 (41.95 - 69.45)	29.74 - 76.4	
Immunosuppressives	Negative	54.05 (42.09 - 59.39)	29.74 - 70.21	NS
	Positive	52.22 (36.89 - 65.27)	31.05 - 86.84	

Table 4. Continued.

		Transferrin receptor		*p*-value
		Median (IQR)	Range	
Mortality	Alive	51.26 (42.27 - 63.8)	34.27 - 70.21	NS
	Died	53.97 (36.86 - 62.49)	29.74 - 86.84	
Severity	Very severe	34.27 (31.05 – 45.41)	29.74 – 68.41	0.004
	Severe	55.15 (48.78 – 63.88)	36.32 – 86.84	
Response to treatment	Yes	55.15 (47.36 – 65.35)	34.02 – 86.84	NS
	No	48.26 (34.62 – 60.39)	29.74 – 70.21	
Infections	Yes	49.41 (38.00 – 59.40)	31.05 – 70.21	NS
	No	54.16 (45.41 – 62.49)	29.74 – 86.84	
Viral markers	Negative	53.1 (38 - 62.49)	29.74 - 86.84	NS
	HCV	46.17 (46.17 - 46.17)	46.17 - 46.17	

$p > 0.05$ is not significant (NS).

Discussion

Aplastic Anemia (AA) is a life-threatening bone marrow failure disease in which allogeneic bone marrow transplantation is the only curative therapeutic option. Decrease in the sTfR levels leads to reduction in erythropoietic activity and minimal iron utilization. This process results in precipitation of iron in the form of ferritin which stimulates the production of hepcidin and dysregulation of iron homeostasis. Increased iron in the marrow leads to limitation in intrinsic hematopoiesis by increasing in the oxidative stress.[7,10]

In this prospective study, we investigated the level of sTfR) in adult Egyptian aplastic anemia patients and its correlation to the severity of the disease and the response to treatment. The sTfR concentration was lower in AA patients in comparison to the normal controls. This observation matched the results reported by the study of Kuiper–Kramer et al., 1996.[11] These data also agreed with the findings of Kuiper–Kramer et al., 1996, who investigated the expression of TfR in patients with anemia of different etiology[11]. The study demonstrated that the expression of TfR was decreased in dysplastic erythropoiesis and aplastic anemia in contrary to iron deficiency, this was associated with an elevated TfR concentration.[12]

There was no significant correlation found in our study between sTfR and TLCs, HBG, platelets count and ferritin level. However, the study by Metzgeroth et al., 2007, showed that the serum concentration of the sTfR is a valuable parameter of erythropoiesis.[13]

Our study results were in line with findings of the study by Yang et al*.,* 2013. Both studies reported that no significant correlation was demonstrated between sTfR and the age of the study patients.[11.14]

In our study, serum levels of sTfR in VSAA patients were significantly lower than in SAA patients which acts as indicator of severity of bone marrow failure in aplastic anemia and the degree of the ineffective erythropoiesis. This result was consistent with finding of other studies done by Yang et al., 2014 and Metzgeroth et al., 2007, that investigated the relationship between levels of sTfR and the severity of aplastic anemia.

In our study, the pretreatment levels of sTfR in responders to immunosuppressive treatment was not different than non-responders. In contrary to our results, the study by Yang et al.,

2013, reported significant higher level of sTfR in responders than non-responders (p=0.005).

In assessing the relation between sTfR level and the response to treatment, 32 patients received immune-suppressive treatment (IST) in the form of cyclosporine 5 mg/kg/day and ATG 5 mg/kd /day for 5 days, of whom 15 had complete response to treatment after 6 months and only three patients were on supportive transfusion on demand according to their CBC. Although the median level of sTfR (nmol/l) in responders was higher than in non-responders, no statistical significance was recorded. This was not in concordance with the finding of a study by Yang WR et al., 2014, which demonstrated that the responders, especially at 3 months, had significantly higher pre-IST baseline of sTfR than that of non-responders (p=0.005), and concluded that sTfR could predict early response to IST therapy in aplastic anemia.[14]

In the present study, as regard the mortality rate due to the AA disease, 65.7% of the patients (n=23) died and 34.35 (n=12) remained alive at the end of the study. About 60% (n=21) of deaths were due to various types of infection, commonly bacterial, viral or fungal, and almost all of them died of septicemia.

In conclusion, the sTfR concentration reflects the remaining erythropoietic activity in aplastic patients and could be an indicator of severity of bone marrow failure. In addition, it may be used to predict the short-term efficacy of IST in AA patients.

Author Contributions

All authors contributed to the study conception and design. Data collection was performed by FSK and RGA. Material preparation, data analysis was performed by FSK and RGA and DMA. The first draft of the manuscript was written in collaboration between by HSM and RGA and DMA. All authors commented on previous versions of the manuscript and approved the final manuscript.

Declaration of Conflicting Interests

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Ethical approval

The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Ain Shams University (FMASU MD 24 /2022).

Informed consent

An informed verbal consent was obtained from each patient and control before being included in the study.

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