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The relation between interleukin-6 and interleukin-8 serum levels and the severity of acquired aplastic anemia in adult patients: A single center study

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Abstract

Aplastic anemia is a lethal bone marrow disease with a heterogeneous etiological background. Interleukin-6 (IL-6) and IL-8 were shown to affect the proliferation and differentiation of primitive hematopoietic cells. They may serve as potential markers for the assessment of severity/prognosis of aplastic anemia. The study aimed to evaluate the levels of IL-6 and IL-8-in patients with aplastic anemia and their relation to disease severity. This study included a total of 35 cases of aplastic anemia, and 27 normal subjects as controls. Levels of IL-6 and IL-8 were quantitatively measured by ELISA. The median serum IL-6 and IL-8 levels in aplastic anemia cases were 125.2 ng/l and 320 ng/l, respectively. These levels were significantly increased in the aplastic anemia patients than in the controls, as the median serum IL-6 was 29.7 ng/l and the median serum IL-8 97ng/l in the controls ($p< 0.001$). A significant correlation was observed between levels of both IL-6 and IL-8 and the severity of the disease ($p<0.001$). In conclusion, IL-6 and IL-8 serum levels are higher in patients with aplastic anemia and have a correlation to the severity of the disease.

Keywords: Aplastic anemia, interleukin-6, interleukin-8, disease

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Introduction

Aplastic anemia (AA) is a life-threatening disease concerning the inability of bone marrow to adequately provide blood cells. It is primarily caused by apoptosis/cell death of hematopoietic progenitors resulting in bone marrow hypocellularity and reduction of hematopoietic cells leading to pancytopenia and reticulocytopenia.[1]

Aplastic anemia ranges in severity from mild to moderate chronic pancytopenia to complete hematological collapse. In 1976, the modified Camitta criteria was developed and are still used to classify AA into three categories based on bone marrow cellularity, reticulocyte count, absolute neutrophil count and platelet count.[2, 3]

Normal hematopoiesis relies heavily upon cytokines and chemokines to maintain effective hematopoiesis and stabilize the hematopoietic microenvironment.[4] Among those cytokines are interleukin 6 (IL-6) and IL -8, which are pleiotropic cytokines with a wide range of biological activities. They are produced mainly by T and B lymphocytes, monocytes and fibroblasts. Recent studies demonstrated that both IL-6 and IL-8 are involved not only in the progress of the stress reaction, inflammatory, and neoplastic diseases of the body but also in regulation of the proliferation and differentiation of primitive hematopoietic cells. Thus, considered potent inhibitors of hematopoiesis with noted effect on tissue damage.[5–7]

Higher levels of IL-6 and IL-8 in both children and adults diagnosed with AA were correlated with disease severity [8, 9.] This further established their role in AA and highlighted their potential use as markers of severity and prognosis in AA patients. The study aimed to evaluate the levels of IL-6 and IL-8-in AA patients and their relation to disease severity.

Subjects and Methods

This case control study included 35 randomly selected patients diagnosed with AA at the Department of Haematology inpatient and outpatient clinics during the period between March 2023 and March 2024. The diagnosis of AA was done according to the International Agranulocytosis and Aplastic Anaemia study in 1987. Patients were divided according to Camitta criteria into mild, moderate and severe AA groups. In addition, 27 apparently healthy volunteers with matched age and sex to the patient group were included as a control group.

Patients with inherited bone marrow failure syndromes, patients with secondary bone marrow infiltration, and patients presenting with secondary aplastic anaemia to radiotherapy or chemotherapy were excluded from the study.

Study procedures

All AA patients were evaluated by careful history taking and examination with emphasis on history of drug intake, radiation and toxic chemical exposure. Laboratory data for the study patients were collected from patients' files at the hospital. These data included: complete blood count, renal function tests, liver function tests, autoimmune markers, and viral markers for Hepatitis C virus. Also, all patients underwent a bone marrow aspiration and biopsy as part of the aplastic anaemia workup.

Serum IL-8 and IL-6 levels were quantitatively estimated by the enzyme linked immunosorbent assay (ELISA) using commercial kits. ELISA kits (IL-8 (E0089Hu) and IL-6 (E0090 Hu) 2021, Biotech Co., Ltd, Shanghai Korean) with colorimetric output were used to determine the plasma concentrations, according to the manufacturer's guidelines. The ELISA plates were read at 450 nm using an automated micro plate reader (iMARK® Microplate absorbance reader Biorad® California, USA).

After obtaining the absorbance values of IL-8 and IL-6 of study samples, Log/log paper was used to determine the concentration of the serum cytokines levels. The log/log curve was plotted against standard absorbance values. Kit sensitivity for IL-6 was 1.03 ng/L and for IL-8 was 2.51 ng/L. Any results above linearity (1000 ng/L) were diluted and results obtained by multiplication with dilution factor.

Statistical Analysis

The data were analysed employing Microsoft Excel 2010 (Microsoft Corporation, Seattle, WA, USA) and the Statistical Package for Social Sciences version 23 (IBBM. Chicago, IL, USA). The quantitative variables are presented, if parametric, as mean, standard deviations and ranges and if non-parametric, as median and interquartile range. Qualitative variables were 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 statistically significant.

Results

The gender and age were not different between AA patients and controls ($p>$ 0.921 and $p>$ 0.740, respectively) as seen in Table 1.

Table 1. Demographic data of the studied patients and the control group.

		Patients group No. = 35	Control group No. = 27	*p*-value
Age	Mean ± SD	35.63 ± 15.23	34.33 ± 15.08	NS
	Range	17 – 66	17 – 58	
Sex	Female	16 (45.7%)	12 (44.4%)	NS
	Male	19 (54.3%)	15 (55.6%)	

p > 0.05 is not significant (NS).

The AA patients were divided according to the severity, following the International Agranulocytosis and Aplastic Anemia Study Group criteria, into two groups: 28 (80%) patients with very severe aplastic anemia (VSAA) and 7 (20%) patients with severe aplastic anemia (SAA). There were no patients in the non-severe group.

There was a statistically significant increase in both IL-6 and IL-8 levels in the patients' group when compared to controls (p <0.001), as shown in Table 2. The median IL-6 level in the patients' group was 125.2 ng/l with interquartile range (IQR) (80.59 – 152.0 ng/l). While the median IL-8 level in the patients' group was 320 ng/l with IQR (245.6 – 461 ng/l). Levels were significantly lower in controls in which the median level of IL-6 was 29.7 ng/l with IQR (23.43 – 35.34 ng/l) and the median level of IL-8 was 97 ng/l with IQR (83 – 118 ng/l), data are shown in Table 2 and Figures 1 and 2.

Table 2. Comparison of interleukin 6 (IL-6) and IL-8 levels between studied patients and the control group.

		Patients group No. = 35	Control group No. = 27	*p*-value
IL-6 (ng/l)	Median (IQR)	125.2 (80.59 – 152.0)	29.7 (23.43 – 35.34)	<0.0001
	Range	51.04 – 245.2	17.54 – 70.92	
IL-8 (ng/l)	Median (IQR)	320 (245.6 – 461)	97 (83 – 118)	<0.0001
	Range	165 – 878	59 – 208	

IL, Interleukin; IQR, Interquartile range. $p \leq 0.05$ is significant.

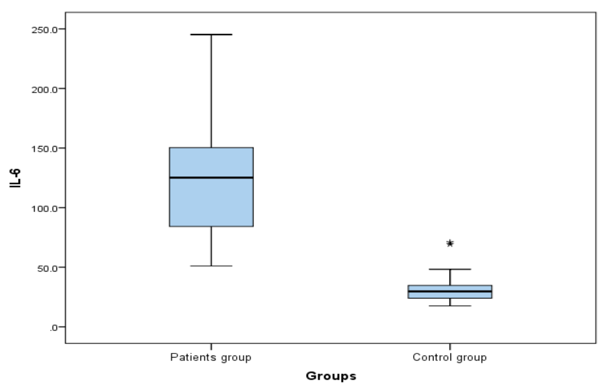

Figure 1. Comparison between interleukin 6 (IL-6) levels (ng/l) in the patient's and control groups.

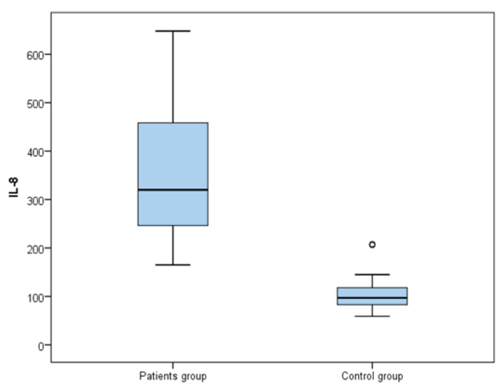

Figure 2. Comparison between IL-8 levels (ng/l) in the patient's and the control group.

A significant negative correlation was observed between both IL-6 and IL-8 (ng/l) and hemoglobin (Hb, g/dl), total leucocytic count (TLC) (10^3/cmm) and platelet count (10^3/cmm) (p<0.001 for all 3 parameters) as shown in Table 3.

Table 3. Correlation between interleukin 6 (IL-6) and IL-8 levels and hemoglobin, total leukocytic count and platelets.

	IL-6		IL-8	
	r	*p*-value	r	*p*-value
TLC (10^3/cmm)	-0.704	<0.0001	-0.702	<0.0001
HGB (g/dl)	-0.731	<0.0001	-0.713	<0.0001
PLT (10^3/cmm)	-0.745	<0.0001	-0.743	<0.0001

IL, Interleukin; TLC, Total Leukocytic Count; HGB, Hemoglobin; PLT, platelets. $p \leq 0.05$ is significant.

The median levels of IL-6 in the VSAA and SAA groups were 132.80 ng/l with IQR (112.50 – 158.80 ng/l) and 72.49 ng/l with IQR (58.09 – 80.59 ng/l), respectively. These levels showed a strong correlation with increasing the severity of the disease (p<0.001) (Table 4).

Similarly, there was a statistically significant relation between the levels of IL-8 and the severity of AA (p<0.001) as the median of IL-8 levels in the VSAA and, SAA, groups were 365.5 ng/l with IQR (256.0 – 513.5 ng/l) and 206.0 ng/l with IQR (177.0 – 271.0 ng/l), respectively (Table 5).

A statistically significant relation was found between levels of both IL-6 and IL-8 and the gender (p <0.05). As the median of IL-6 was 143.35 ng/l (125.95 - 153.4 ng/l) in females and 107.1 ng/l (69.2 - 127.6 ng/l) in males. While the median level of IL-8 was 378 ng/l (295.95 - 489.5 ng/l) in females and 254 ng/l (206 - 461 ng/l) in males (Table 4 and 5).

Table 4. Relation of interleukin 6 (IL-6) with the other studied parameters among the patients' group.

		IL-6		*p*-value
		Median (IQR)	Range	
Sex	Female	143.35 (125.95 - 153.4)	63.76 - 222.5	0.034
	Male	107.1 (69.2 - 127.6)	51.04 - 245.2	
Severity	Severe aplastic anemia	72.49 (58.09 – 80.59)	51.04 – 87.84	0.001
	Very Severe aplastic anemia	132.80 (112.50 – 158.80)	63.76 – 245.20	

IL, Interleukin; IQR, Interquartile range. $p \leq 0.05$ is significant.

Table 5. Relation of interleukin 8 (IL-8) with the other studied parameters among the patients' group:

		IL-8		*p*-value
		Median (IQR)	Range	
Sex	Female	378 (295.95 - 489.5)	207.9 – 878	0.047
	Male	254 (206 - 461)	165 – 782	
Severity	Severe aplastic anemia	206.0 (177.0 – 271.0)	165.0 – 327.0	0.002
	Very Severe aplastic anemia	365.5 (25 6.0 – 513.5)	206.0 – 878.0	

IL, Interleukin; IQR, Interquartile range. $p \leq 0.05$ is significant.

Discussion

This was a case control study in which we investigated the expression of both serum IL-6 and IL-8 in adult patients with AA and compared them to a control group as well as studying the relation between those levels and other clinical and laboratory parameters.

In our present study, AA patients had significantly higher levels of both IL-6 and IL-8 when compared to the control group (*p*<0.001, for both, see Table 2). This agreed with findings of studies by Gupta et al., 2017, Bhargawa et al., 2022 and Singh et al., 2022 who evaluated IL-6 and IL-8 levels in patients with AA.[8-10]

The research by Gupta et al., 2017[8] and Singh et al., 2022[10] studied AA in a pediatric patient population where the mean age was 9.78±2.74 years and 11.3 ± 3.7 years, respectively. They found that IL-6 levels in patients were higher compared with controls (193.48±352.3 ng/l vs. 4.58±3.39 ng/l, *p*<0.001) and (278.88 ± 216.03 ng/l vs. 4.51 ± 3.26 ng/l; *p*< 0.001), respectively. While IL-8 levels were also higher compared with controls (15.58±18.0 ng/l vs. 1.85±0.95 ng/l; *p*<0.001) and (20.28 ± 94.98 vs. 1.79 ± 0.78 ng/l; *p* < 0.001), respectively.

The research by Bhargawa et al., 2022[9] studied AA in adult patients with mean ages of 21.31±16.41 years. They reported that levels of IL-6 in patients was higher when compared to controls (283.28±220.27 ng/l vs. 7.52±1.43 ng/l, *p*<0.001) and also IL-8 levels were similarly higher when compared with controls levels (122.56±97.79 ng/l vs. 3.42±1.73 ng/l; *p*<0.001).

When assessing the severity of AA against blood levels of IL-6 and IL-8, levels of both interleukins were significantly higher in individuals with VSAA (p=0.001). In comparing the median of IL-6 and IL-8 levels in a relation to the severity of AA, and it was observed that their levels displayed a significant incremental trend with increased severity of AA (*p*<0.001). Such data are consistent with those presented in studies done by Gupta et al., 2017, Bhargawa et al., 2022, Singh et al., 2022, and Tripathy et al., 2005 who all investigated the relationship between IL-6 and IL-8 levels in AA patients and disease severity [8-11.]

Tang X et al.,1998 studied IL-8 only while Salama et al., 2022 studied IL-6 only, both studies observed higher levels of the studied interleukins, they were significantly correlated with the severity of AA.[5, 12]

Our study results and the agreement with data of other mentioned studies imply that patients with AA have probably an inflammatory background. And that this underlying inflammatory process may be exaggerated in patients with very severe AA.

In our study, a significant negative relation was demonstrated between both IL-6 and IL-8 (ng/l) and Hb (g/dl), TLC (10^3/cmm) and platelet count (10^3/cmm) (p<0.001 for all 3 parameters). These were also seen in the research by Salama et al., 2022 who studied only Il-6 and showed negative relation between IL-6 and HB and TLC.[5]

According to these findings, IL-6 and IL-8 most probably have a strong inhibitory influence on the bone marrow microenvironment. This is reflected in the peripheral blood complete blood count values as was mentioned by Tripathy et al., 2005, who stated that IL-8 can suppress hematopoiesis through inhibiting the proliferation of myeloid progenitors by transducing an inhibitory signal via receptor–ligand-mediated mechanism.[11]

The limitations of our study are the small number of cases, and that they were from a single center, and the lack of patient follow-up. Further multi-institutional investigations involving higher number of cases and centers would be insightful into the significance of IL-6 and IL-8 levels in AA patients.

In conclusion, levels of IL-6 and IL-8 are elevated in patients with AA and have a significant correlation with the severity of the disease. Their increasing levels may play a role in implementation of the appropriate regimen to stop further progression of the disease.

Author Contributions

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

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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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 (approval #: FMASU R271/2022/2023).

Informed consent

All the procedures were explained to study subjects, each participant provided a verbal informed consent.

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