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Serum level of interleukin: 8 in correlation with aplastic anemia severity

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Abstract
Background: Aplastic anemia (AA) is a disease in which the bone marrow (BM) and the hematopoietic stem cells are damaged causing pancytopenia. It may be hereditary or acquired and it can range from mild to severe. Interleukin-8 (IL-8) is a chemokine that is critical for tissue damage in acute inflammation and has been shown to be potent inhibitors of myelopoiesis. Interleukin promotes macrophages to produce IL-8 in AA. The aim of the present work was to evaluate the level of IL-8 in patients with AA and its correlation with severity of the disease.
Methods: The present study was conducted on 40 individuals, 30 newly diagnosed AA patients and 10 healthy subjects served as control. They are classified to group I (8 patients with very severe AA 20%), group II (16 patients with severe AA 40%), group III (6 patients with non-severe AA 15%) and group IV (10 apparently healthy individuals as control). All groups were subjected to complete blood count (CBC), BM aspiration and biopsy for patients only, erythrocyte sedimentation rate (ESR) and lactate dehydrogenase (LDH), measuring level of serum IL-8 of AA patients by enzyme-linked immunosorbent assay (ELISA).
Results: Serum levels of IL-8 were significantly higher in AA patients at diagnosis when compared to the levels in normal controls. A significant negative correlation was found between serum level of IL-8 and Hb level, platelets count, neutrophil count, reticulocyte count and BM cellularity, also a significant positive correlation was found between serum level of IL-8 and ESR.
Conclusion: Level of serum IL-8 is higher in very severe AA patients than severe AA patients than non-severe AA patients, that suggested it may be involved in the mechanisms of AA hence it correlates with the disease progression.

Keywords: Aplastic anemia, interleukin-8, bone marrow

Introduction

Toxins, radiation, chemotherapy, medicines, infectious diseases, and autoimmune diseases can all lead to aplastic anaemia (AA), a rare disease characterised by a deficiency of all three blood cells (pancytopenia), [red blood cells (RBCs) (anaemia), white blood cells (leukopenia), and platelets (thrombocytopenia)]. In roughly 50% of instances, the underlying reason has not been determined [2].

Levels of AA severity vary. The diagnosis of AA requires a sample of hypocellular bone marrow [3]. Severe AA defined as either a bone marrow cellularity of 25% or a cellularity of 50% with 30% leftover blood cells with at least 2 of the following; Absolute reticulocyte count 20 109/L (60 109/L using automated - analysis), platelet count 20 109/L, and absolute neutrophil count 0.5 109/L; very severe AA when absolute neutrophil count 0.2 109/L and fulfils rest of the criteria for severe AA; and non-severe AA when patients do not meet the criteria for sever or very severe AA [4].

One class of cytokines known as interleukins (ILs) has many roles in modulating the immune system. Macrophages, along with epithelial cells, airway smooth muscle, and endothelial cells, produce IL-8, a chemokine that plays an important role in cell differentiation and activation [5]. Target cells' chemotaxis is stimulated by IL-8, prompting them to migrate towards the site of infection [6].

Imbalances in the immune system are thought to play a significant part in AA's unclear aetiology. Interferon c and tumour necrosis factor (TNF) are examples of T helper type I cytokines that, when produced in excess, have been linked to the onset of AA [7]. In addition, IL-6 is a pleiotropic cytokine with numerous biological functions, such as the differentiation and activation of T cells and macrophages, as well as the terminal differentiation of B cells [8].

Macrophages, neutrophils, T cells, fibroblasts, etc., are just some of the many cell types that produce IL-8. To a large extent, they inhibit myelopoiesis, which is why they play such a crucial role in tissue damage during acute inflammation. Macrophages in AA are stimulated to generate IL-8 by interleukin [9].

In AA cases, IL-8 levels, which are generated by macrophages, were found to be significantly higher than in healthy individuals. The production of IL-17A mRNA was found to be significantly higher in both bone marrow mononuclear cells and peripheral blood mononuclear cells from an AA patient. Both bone marrow and peripheral blood plasma IL-17 levels were found to be higher in AA patients compared to healthy subjects. Macrophages from both AA patients and healthy subjects responded to L-17 by producing IL-6 and IL-8. Similarly, TNF- was produced in response to IL-17 activation. According to the findings, IL-17 and IL-17-induced IL-6, IL-8, and TNF- may play a role in the pathogenesis of AA [10].

The purpose of this study was to assess IL-8 levels in AA patients and examine their association with disease symptoms.

Patients and Methods

This present study was conducted on 40 individuals, 30 newly diagnosed AA patients attending to outpatient clinic in Tanta University Hospital before starting treatments and 10 healthy subjects served as control.

The study was done after approval from the Ethical Committee Tanta University Hospitals. An informed consent was obtained from all participants in the research controls were volunteers.

Exclusion criteria were pancytopenia due to causes other than AA, patients under immune suppressive therapy, other causes of pancytopenia such as malignancy (except hypoplastic and aplastic malignancy) and hypersplenism, drugs causing aplasia such as cytotoxic drugs.

Patients were further subdivided into group I: 8 patients with very severe AA at time of diagnosis, 5 males and 3 females with age ranged between 12-42 years, group II: 16 patients with severe AA at time of diagnosis, 10 males and 6 females with age ranged between 4-62 years, group III: 6 patients with non-severe AA at time of diagnosis, 4 males and 2 females with age ranged between 6-41 years and group IV: 10 healthy individuals, 7 males and 3 females with age ranged between 7-41 years as a control group.

All groups were subjected to history taking and careful clinical examination, routine laboratory investigations (CBC which was done on ERMAPCE- 210 N cell counter, bone marrow aspirations and biopsy for patients only, erythrocyte sedimentation rate (ESR), serum LDH determination by dinitrophenyle hydrazine method), specific laboratory investigation such as reticulocyte count, measuring level of serum IL-8 of AA patients by enzyme-linked immunosorbent assay (ELISA), bone Marrow aspiration and biopsy and measuring serum level of IL-8.

Peripheral blood sample: 8 ml of venous blood were withdrawn from anti cubital vein of each subject by the use of disposable sterilized plastic syringe and were divided as follows: 2 ml of blood was put on ethylenediaminetetraacetic acid (EDTA) (1mg/ml blood) and mixed thoroughly to perform complete blood picture done on ERM APCE – 210 N cell counter, 1.6 ml was added to a tube containing sodium citrate for ESR and 4 ml was collected in the plain voluntainer tube, allowed to clot, to separate serum for LDH examination.

CBC to check hemoglobin concentration, platelets count and white blood cells count, performed conventionally and by electronic automatic cell-counter (ERMA PCE -210 cell counter). Peripheral blood smears were stained with Leishman's stain and for detection of blast cells and their percent [11].

Erythrocyte sedimentation rate (ESR): Anticoagulated blood was placed in an upright westergren tube, and the rate of RBCs sedimentation was measured and reported in mm/h [12].

Serum lactate dehydrogenase (LDH): [13] which is useful in diagnosing tissue damage; it catalyzes the reversible reduction of pyruvate to lactate using NADH. The oxidation of NADH, which is monitored by reflectance spectrophotometry, is used to measure lactate dehydrogenase activity.

Reticulocyte count: A blood sample is combined with dye solution and placed in a test container. Put the cap on the tube and put it in an incubator at 370 degrees for 10 to 15 minutes. After incubation, blend to create a discoloured blood film. When the pictures are ready, you can look at them without fixing or counterstaining them. Try to determine how many reticulocytes are present in a sample of 1,000 red blood cells. Reticulocytes will display a dark-blue reticulum or network [14].

Bone marrow sample

Bone marrow aspiration was done from anterior iliac spine under complete aseptic technique. Film preprinted, stained with Gimesa stain form morphological study, as well as cytochemical stains. The sample was drawn into EDTA tube for total count and for immunophenotyping study.

Local anaesthesia (1% plain lidocaine), a 25-gauge needle with two 5-ml vials, and clean drapes and mittens were all part of the equipment list. If chlorhexidine was prohibited, povidone-iodine could have been used instead, and an 11-blade scalpel for a Tools needed for a trephine biopsy include two bigger needles (10 ml or 35 ml), a throwaway bone marrow aspiration needle, a Jamshidi bone marrow biopsy needle, slides, and a preservative-free Heparin sulphate solution. In order to lessen the pain of a local anaesthesia infusion, sterile sodium bicarbonate can be added to the anaesthetic.

The patient should be given a sufficient quantity of local anaesthesia for the discomfort they will experience during the bone marrow aspiration and biopsy. Administering anaesthesia to children requires a doctor or other trained professional.

Patient or legal caretaker written approval was received. Check the patient's identification to make sure we're operating on the right person.

Bone marrow aspiration

After administering local anaesthetic, we made a small cut to insert the bone marrow aspiration syringe, which was inserted at a right angle to the iliac crest. As soon as the needle penetrated the marrow chamber, it remained stationary without further assistance. Once the periosteum

has been broken, the needle should be pushed forward through the cortex while being rotated in a semicircle, first in a clockwise direction and then in the opposite direction. As soon as the needle was firmly embedded in the bone. Once the stylet was out, 1 millilitre of pure bone marrow was aspirated into the tube. It was decided to leave the needle in position and continue filling in order. Sample-withdrawal syringes that have been prepped with heparin or other anticoagulants or stabilisers, as needed by individual studies. After reattaching the hypodermic to the needle or re-inserting the stylet, we took the needle out.

Bone marrow biopsy

A second core biopsy was performed through the same skin cut, with a slightly angled needle entry into the bone. The periosteum was pierced by inserting the needle with the stylet facing backward. After withdrawing the stylet, you should move the needle in a semicircle or circle to a distance of 2 centimetres. Once the needle has been removed, the core can be retrieved by using a thinner, sterile stylet or probe to force the material from the needle point out the proximal end of the needle and onto sterile gauze or a plate. Touch preparation slides were made from the biopsy material right there in the hospital, and they were stained with hematoxylin and eosin (H&E). The slides were then identified and stored in a sterile receptacle.

When sufficient hemostasis was achieved through pressure, the region was disinfected with alcohol or another suitable agent. Compression bandaging was used to apply clean or antibiotic-soaked bandages to the wound. In 24 hours, the patch can be taken off. The site should be observed for signs of infection or prolonged haemorrhage after the gauze is taken off [15].

Measuring serum level of IL-8 [16]

Test principle

Using a double - antibody sandwich ELISA to determine the IL-8 concentration in blood samples (ELISA). Incubate IL-8 in a monoclonal antibody enzyme well that has been pre-coated with a human IL-8 monoclonal antibody, add biotin-labeled anti-IL-8 antibodies that have been mixed with Streptavidin-HRP to create an immune complex, incubate again to eliminate any unbound enzyme, and repeat. After adding Chromogen Solution A,B, the liquid turns blue, and a positive correlation is observed between the quantity of the human compound IL-8 in the sample and the colour change.

Reagent

Reagent for human IL-8 ELISA: 0.5ml of standard (320 ngIL), 3ml of standard diluents, micro ELISA Strip plate (12well X 8strips), 6ml of str-HRP-Conjugate Reagent, 20ml of 30X wash solution 1ml of biotin IL-8 Ab, 6ml of chromogen Solution A, 6ml of chromogen Solution B and 6ml of stop Solution. We also used 37 C incubator, standard enzyme reader, precision pipettes and disposable pipette tips, distilled water, disposable tubes for sample dilution and absorbent paper.

Assay procedure

1. Standard dilution the original standard reagent was diluted as follows
2. Inject samples

160 ng/liter	Standard No. 5	120 1 Original Standard + 120 - 1 Standard diluent
160 ng/liter	Standard No. 4	120 1 Standard No. 5 + 120 1 Standard diluent
160 ng/liter	Standard No. 3	120 1 Standard No. 4 + 120 v 1 Standard diluent
160 ng/liter	Standard No. 2	120 » 1 Standard No. 3 + 120 » 1 Standard diluent
160 ng/liter	Standard No. 1	120 - 1 Standard No. 2 + 120 - 1 Standard diluent

Blank preparing

The blank wasn't prepared by adding samples and IL 8 – antibody labeled with biotion, streptavidin - HRP, only chromogen solution is allowed.

Standard preparing

After adding the standard 50 N1, streptavidin - HRP 50 N1, sample 40 N1, and IL-8 antibody 10 N1, the membranes were sealed, lightly shook, and kept at 37 c for 60 minutes. The 30x cleaning concentrate is diluted with 30 times as much purified water as a backup. Cleaning up after a meticulous removal of the membrane, during which time water was emptied and discarded. Following the addition of 50 ml of chromogen solution A and 50 ml of chromogen solution B to each well, gentle mixing, and incubation at 37 C in the dark for 10 minutes ensued. Each well had 50 ml of stop solution introduced to it to halt the process (the blue changes into yellow immediately).

Within 15 minutes of applying the stop solution, the optical density (OD) under 450 nm wavelength should be recorded, with the blank well serving as the standard. The linear regression equation for the standard curve was computed, and then the OD readings of the sample were applied to the regression equation to determine the appropriate concentration.

Statistical analysis

For the statistical study, we used SPSS v26 (IBM Inc., Chicago, IL, USA). We used analysis of variance (ANOVA) (F) test with post hoc test (Tukey) to evaluate the two groups on quantitative variables given as means and standard deviations (SDs). Qualitative variables were presented as frequency and percentage (%) and were analyzed utilizing the Chi-square test. Evaluation of diagnostic performance sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). The overall diagnostic performance of IL-8 was assessed by ROC curve analysis. A two tailed P value < 0.05 was considered statistically significant.

Results

There was statistically significant increase among studied groups regarding pallor, purpura and fever ($p<0.001$). Table 1.

Table 1: Clinical manifestations of studied groups (n = 40)

Clinical findings	Groups				P-value
	Group I	Group II	Group III	Group IV	
Pallor	8 (100%)	3 (18.75%)	0 (0%)	0 (0%)	<0.001*
Purpura	8 (100%)	1 (6.25%)	0 (0%)	0 (0%)	<0.001*
Fever	6 (75%)	2 (12.5%)	0 (0%)	0 (0%)	<0.001*

Data are presented as frequency (%), * significant as P value < 0.05.

A significant decrease in Hb between (II & I), (II & III), (II & IV), (I& III), (I & IV) and (III & IV) (p<0.05). A significant decrease in platelets, neutrophils, reticulocytes between (II & III), (II & IV), (I & III), (I & IV) and (III & IV) (p<0.05), but there was non-evident variation between (II & I). A significant increase in ESR between (II & I), (II & III), (II & IV), (I & III), (I & IV) and (III & IV) (p<0.001), but there was statistically non-evident variation between (III&IV). There was statistically significant decrease between (I &II), (I&III) and between (II &III) (p<0.05). There was non-evident variation in LDH between the studied groups. Table 2.

Table 2: Comparison between mean values of Hb, Platelets, Neutrophil, Reticulocytes, LDH, ESR and Cellularity in studied groups

	Group I	Group II	Group III	Group IV	P-value	TUKEY'S Test
Hb (g/dl)	7.413±0.603	8.231±0.535	9.267±0.388	12.420±0.719	<0.001*	I&II=0.013* I&III<0.001* I&IV<0.001* II&III=0.004* II&IV<0.001* III&IV<0.001*
Platelets (x10³/cm mm)	8.889±2.473	14.068±3.142	91.718±4.085	378.000±31.552	<0.001*	I&II=0.877 I&III<0.001* I&IV<0.001* II&III<0.001* II&IV<0.001* III&IV<0.001*
Neutrophil (x10³/cm mm)	0.123±0.023	0.281±0.105	1.067±0.266	3.700±0.981	<0.001*	I&II=0.156 I&III<0.001* I&IV<0.001* II&III<0.001* II&IV<0.001* III&IV<0.001*
Reticulocytes%	0.263±0.106	0.263±0.102	0.667±0.225	1.130±0.467	<0.001*	I&II=1.000 I&III=0.033* I&IV<0.001* II&III<0.001* II&IV=0.013* III&IV=0.008*
LDH (U/L)	166.250±30.179	162.625±30.919	164.333±41.113	154.000±31.927	0.858	--
ESR (mm/h)	129.500±19.442	73.375±11.448	29.167±3.971	18.700±3.268	<0.001*	I&II<0.001* I&III<0.001* I&IV<0.001* II&III<0.001* II&IV<0.001* III&IV=0.310
Cellularity%	10.125±3.044	21.438±2.159	22.67±1.75	--	<0.001*	I&II<0.001* I&III<0.001* II&III=0.031*

Data are presented as mean±SD, *significant as P value < 0.05. Hb: Hemoglobin, LDH: Lactate dehydrogenase, ESR: Erythrocyte sedimentation rate.

As regard to cellularity of BM aspiration, in group (I) BM aspiration was hypo cellular in one patient (12.50%), bloody in 2 cases (25%) and dry tap in 5 patients (62,50%). In group (II), BM aspiration was hypo cellular in all 16 patients (100%), in group (III) all 6 patients (100%) were hypo cellular. BM biopsy was hypocellular in all cases. It was mild hypocellular in 6 cases out of 30 (20%), moderate hypocellular in16 cases (54%) and severe hypocellular in 8 cases (26%), in 22 cases (74%), BM biopsy was hypocellular with marked decrease in hematopoietic cells (decrease erythropoiesis, granulopioesis and thromopioesis) which replaced by fat cells. Non hematopoietic cellularity represented by macrophages, lymphocytes and plasma cells. There was increase in mast cells esinophils, fibrosis and stromal oedema. In 6 cases (18%), BM biopsy was hypocellular with predominance adipocytosis. In other 2 cases (8%), BM biopsy was acellular. A significant increase in serum level of IL-8 between (II & I), (II & III), (II & IV), (I & III), (I & IV) (p<0.001), but there was non-evident variation between (III & IV). Table 3

Table 3: Comparison between cellularity of BM aspirate and serum level of IL-8 (pg/ml) between studied groups

	BM Aspirate	Group I	Group II	Group III	Group IV	P-value	TUKEY'S Test
BM aspirate	Hypo cellular	1 (12.5%)	16 (100%)	6 (100%)	--	--	--
	Bloody	2 (25%)	0 (0%)	0 (0%)	--		
	Dry tap	5 (62.5%)	0 (0%)	0 (0%)	--		
Cellularity of Biopsy							
Normo cellular		0 (0%)					
Hypo cellular		30 (100%)					
Hyper cellular		0 (0%)					
IL-8 (pg/ml)		**Group I**	**Group II**	**Group III**	**Group IV**	**P-value**	**TUKEY'S Test**

	66.175±3.608	14.569±0.386	1.750±0.599	2.000±0.587	<0.001*	I&II<0.001* I&III<0.001* I&IV<0.001* II&III<0.001* II&IV<0.001* III&IV=0.991

* significant as P value < 0.05. BM: Bone marrow, IL-8: Interleukin-8

Table 4 shows that there was a significant positive correlation was found between serum level of IL-8 and pallor, purpura and fever (p<0.001).

Table 4: Correlation was found between serum level of IL-8 and clinical manifestation in studied groups

		IL-8 (pg/ml)	T-Test	
			t	P-value
Sex	Male (n=19)	24.826±24.776	-0.263	0.795
	Female (n=11)	27.391±27.441		
Pallor	Positive (n=11)	52.182±24.156	7.222	<0.001*
	Negative (n=19)	10.474±6.106		
Purpura	Positive (n=9)	60.411±17.618	11.632	<0.001*
	Negative (n=21)	10.919±5.959		
Fever	Positive (n=8)	53.938±24.366	4.932	<0.001*
	Negative (n=22)	15.523±16.636		

* significant as P value < 0.05. IL-8: Interleukin-8

As regard group (I), a significant negative correlation was found between serum level of IL-8 and Hb level, platelets count & BM cellularity (p<0.05), but non-significant correlation was found between it and age, neutrophil count, reticulocyte count, LDH level and ESR. As regard group (II), there was non-significant correlation was found between serum level of IL-8 and (age, Hb level, platelets count and neutrophil count, reticulocyte count, LDH level, ESR and BM cellularity. As regard group (III), there was non-significant correlation was found between serum level of IL-8 and (age, Hb level, platelets count and neutrophil count, reticulocyte count, LDH level, ESR and BM cellularity. As regard total, a significant negative correlation was found between serum level of IL-8 and Hb level, platelets count, neutrophil count, reticulocyte count and BM cellularity (p<0.05), while a significant positive correlation was found between serum level of IL-8 and ESR (p<0.001). Table 5.

Table 5: Correlation was found between serum level of IL-8 and laboratory findings in studied groups

Correlations								
	IL-8 (pg/ml)							
	Group I		Group II		Group III		Total	
	R	P-value	r	P-value	r	P-value	r	P-value
Age (Years)	-0.349	0.396	0.237	0.378	-0.036	0.946	0.017	0.928
Hb (g/dl)	-0.911	0.002*	-0.470	0.067	-0.456	0.364	-0.715	<0.001*
Platelets (x10^3/cm mm)	-0.741	0.036*	0.269	0.314	-0.076	0.886	-0.540	0.002*
Neutrophil (x10^3/cm mm)	-0.588	0.125	-0.312	0.239	-0.691	0.129	-0.604	<0.001*
Reticulocytes%	-0.555	0.153	-0.065	0.810	-0.660	0.154	-0.450	0.013*
LDH (U/L)	0.626	0.097	-0.352	0.181	0.486	0.329	0.060	0.753
ESR (mm)	-0.075	0.860	0.354	0.178	-0.240	0.648	0.892	<0.001*
Cellularity%	-0.836	0.010*	-0.454	0.077	-0.515	0.295	-0.912	<0.001*

* significant as P value < 0.05. Hb: Hemoglobin, LDH: Lactate dehydrogenase, ESR: Erythrocyte sedimentation rate

Table 6 and figure 1 show that the cutoff is >2.8, the sensitivity is 80.0%, the specificity is 100.0%, PPV is 100.0%, NPV is 62.5 and accuracy (area under the curve) is 86.8%.

Table 6: Diagnostic performance of serum IL-8 between patient and control group

ROC curve between Cases and Control					
Cutoff	Sensitivity	Specificity	PPV	NPV	Accuracy
>2.8	80.0	100.0	100.0	62.5	86.8%

PPV: Positive predictive value, NPV; negative predictive value, ROC: Receiver operating characteristic curve

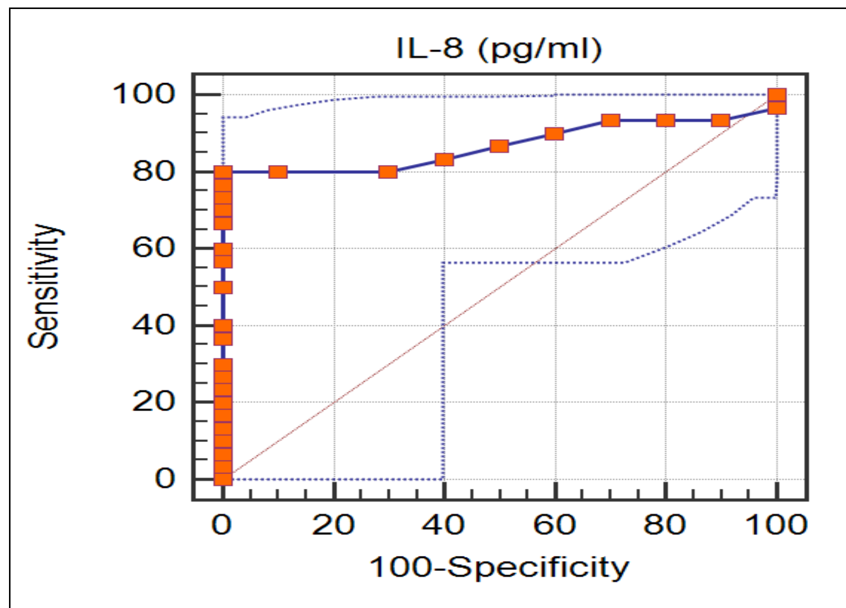

Fig 1: ROC Curve showing the diagnostic performance of serum IL-8 between patient and control group

Discussion

In the present study, as regard to clinical manifestations, pallor was found in all patients 100% in group I, in 3 patients out of 16 in group II (18.75%) and didn't appear in group III and IV, a significant increase among studied groups (*p*< 0.001). Purpura was found in all patients 100% in group I, in one patient out of 16 in group II (6.25%) and wasn't found in group III & IV. A significant increase among studied groups (*p*< 0.001). Fever was found in 6 patients out of 8 in group I (75%), in 2 patients out of 18 in group II (12.50%) and didn't appear in group III and IV. A significant increase among studied groups (*p*< 0.001).

These results agree with Das *et al.* [17] who found that the most common complaints among all patients were fever (61.9% cases), purpura, melena, or per rectal bleeding were the most common bleeding manifestations. Severe pallor was noted in 16 (38.1%) patients, followed by moderate pallor in 14 (33.3%) and mild pallor in 28.6% of cases.

In the present work, as regard to hematological findings, Hb level, a significant decrease in Hb level among studied groups (*p*<0.001) in the patients groups compared to healthy controls, where in group (I) Hb level ranged between 6.4 – 8.1 (g/dl) with a mean of 7.413±0.603, in group (II) Hb level ranged between 7.2-9 (g/dl) with a mean of 8.231±0.535, in group (III) Hb level ranged between 8.8 – 9.8 (g/dl) with a mean of 9.267±0.388 and in group (IV), it ranged between 11.2 – 13.5 (g/dl) with a mean of 12.420±0.719. Also, a significant decrease between (II & I), (II & III), (II & IV), (I& III), (I & IV) and (III & IV) as the P-value is (0.013, 0.001, 0.001, 0.004, 0.001, 0.001) respectively.

This agreed with Gupta *et al.* [18] who reported that the mean of Hb (g/dL) in AA was (3.9×109/L)± (1.86×109/L) (median 3.5×109/L).

The mechanism of decreased Hb level is due to bone marrow hypoplasia that leads to decrease the production of red and white blood cells and platelets (pancytopenia). Having fewer red blood cells causes hemoglobin to drop [19].

In the present study, as regard to platelets count, a significant decrease in platelets count between the studied group (*p*<0.001) as compared to healthy controls where in group (I) platelets count ranged between 5 X10³ -12 X10³/cm mm with a mean 8.889±2.473, in group (II), it ranged between 8 X10³-18 X10³ /cm mm with a mean of 14.068±3.142, in group (III), it ranged between 85 X10 ³ - 97 X 10³ with a mean of 91.718±4.085, in group(IV), it ranged between 320X10³ - 415X10³ with a mean of 378.000±31.552, also a significant decrease between (II & III), (II & IV), (I & III), (I & IV) and (III & IV) (*p*<0.001), but there was non-evident variation between (II & I) as (P = 0.877).

This in agreement with Ashwini *et al.* [20] and Gupta *et al.* [18] who reported that the mean platelet count was 19.17 X10³ ± 15.6 X10³ (median 14.5).

In the present work, as regard to neutrophil count, a significant decrease between studied groups (*p*<0.001), also a significant decrease between (II&III), (I&III), (I&IV), (II&IV) and (III&IV) (*p*<0.001) as compared to healthy controls where in group (I), it ranged between 0.1x10³ - 0.16x10³ (cm-mm) with a mean of 0.123±0.023, in group (II), it ranged between 0.2x10³ - 0.4x10³ (cm mm) with a mean of 0.281±0.105, in group (III), it ranged between 0.8x10³ - 1.4x10³ (cm mm) with a mean of 1.067±0.266 and in group (IV), It ranged between 2x10³ - 7x10³ (cm mm) with a mean of 3.700±0.981. (cm mm), there was non-evident variation between II & III (P = 0.107).

This result agrees with that of Ashwini *et al.* [20] and Gupta *et al.* [18] who found that the absolute neutrophil count ($\times 10^9$/L) was 0.69 ± 0.44 (median 0.62) in cases with AA but not determine its severity and that due to hypoplastic bone marrow and peripheral pancytopenia [21].

In the present study, as regard to reticulocyte count, There was a significant decrease between studied groups (*p*< 0.001), also there was a significant decrease between (II & III), (II & IV), (I & III), (I & IV) (*p*< 0.001) as compared to healthy controls where in group (I), it ranged between 0.1 – 0.4 (%) with a mean of 0.263±0.106, in group (II),it ranged between 0.1 – 0.4 (%) with a mean of 0.263±0.102, in group (III), it ranged between 0.3 – 0.9 (%) with a mean of 0.667±0.225, in group (IV), it ranged between 0.6 – 1.9 (%) with a mean of 1.130±0.467, there was non-evident variation between (II & I) and (III & IV) as P-value was (1.000 and 0.655) respectively, which is in agreement with the Mary *et al.* [22] study. This explained by bone marrow

hypoplasia [23].

In the present work, the results were supported by Marsh *et al.* [24] observation as they concluded Pancytopenia is commonly seen on a complete blood count (FBC), though lymphocyte counts are generally unaffected. In the later stages, mono-cytopenia, especially thrombocytopenia, may appear, but in the early stages, the haemoglobin level, neutrophil count, and platelet count are all equally depressed.

In the present study, as regard to LDH level, there was non-evident variation between the studied groups (P = 0.858), where in group (I), it ranged between 112 – 189 (u/l) with a mean of 166.250±30.179, in group (II), it ranged between 101 – 219 (u/l) with a mean of 162.625±30.919 in group (III), it ranged between 123 – 240 (u/l) with a mean of 164.333±41.113 and in group (IV), it ranged between 107 – 205 with a mean of 154.000±31.927

On the other hands, as regard to ESR, there was statistically significant increase between the studied groups as P- value was < 0.001.

Also a significant increase between (II & I), (II & III), (II & IV), (I & III), (I & IV) and (III & IV) (p<0.001), where in group (I),it ranged between 107 – 165 (mm/h) with a mean of 129.500±19.442, in group (II), it ranged between 58 – 91(mm/h) with a mean of 73.375±11.448, in group (III), it ranged between 24-35 (mm/h) with a mean of 29.167±3.971, in group (IV),it ranged between 13–25(mm/h) with a mean of 18.700±3.268, but there was evident variation between the studied groups (p<0.001).

This result agrees with Kapoor [25], who reported that a case with the ESR was consistently more than 120 mm and hemoglobin only 5-6 gm., with examination of bone marrow aspirate revealed that the patients had AA.

in the present work, as regard to BM cellularity%,a significant decrease between studied groups (p<0.001), where in group (I), it ranged between (5 -14)% with a mean of 10.125±3.044, in group (II), it ranged between (16 -24)% with a mean of 21.438±2.159, in group (III), it ranged between (20 -24)% with a mean of 22.67±1.75 and also a significant decrease between (I&II), (I&III) and (II&III).

This result agrees with Killick *et al.* [26] who put classification of AA according to severity in which BM cellularity>25% (or >50% if >30% of BM is hematopoietic cells. This explained by bone marrow hypoplasia [27].

As regard to BM aspirate, the present study showed that in group (I), one patient out of 8 (12.5%) was hypocellular, two cases out of 8 (25%) were bloody and 5 patient (62.5%) were dry tap, in group (II) all 16 patients were hypocellular and in group (III) all 6 patients were hypocellular.

Aspirate of 5 patients out of 30 (17%) were dry tap aspirate. In another two cases (6%) BM aspirate were non diagnostic because it was bloody. Other 23 aspirates out of 30 (77%) were hypocellular in different degrees with depression of all hematopoietic cells (erythroid, myeloid and megakaryocytes lines) less than 25% with predominance of lymphocytes and plasma cells. There was absence of significant fibrosis or neoplastic infiltration.

In one another case BM aspirate showed hypocellularity predominance with a few normocellular particles (hot spots), but megakaryocytes were reduced.

BM biopsy was hypocellular in all cases. It was mild hypocellular in 6 cases out of 30 (20%), moderate hypocellular in16 cases out of 30 (54%) and severe hypocellular in 8 cases out of 30 (26%). In 22 cases out of 30 (74%) BM biopsy was hypocellular with marked decrease in hematopoietic cells (decrease erythropoiesis, granulopioesis and thromopioesis) which replaced by fat cells. Non hematopoietic cellularity represented by macrophages, lymphocytes and plasma cells. There was increase in mast cells esinophils, fibrosis and stromal oedema. In 6 cases (18%) BM biopsy was hypocellular with predominance adipocytosis. In other 2 cases (8%) BM biopsy was acellular.

These results agree with Lu *et al.* [28] who graded cellularity of bone marrow biopsy into four grades: distinct decrease, extreme decrease, distinct increase, and extreme increase.

Das *et al.* [17] reported that bone marrow aspiration smears and imprint smears differ considerably in their positive predictivity in diagnosing AA. Marrow aspirations can be unsuccessful in marrow failure resulting in dry tap or blood tap. Acellular or paucicellular aspirations can occur in marrow aplasia or packed marrow in leukemic marrow, myelodysplasia, myelofibrosis, etc. Hypocellular marrow aspirations can be met in AA, hypocellular myelodysplastic syndrome, myelofibrosis, and several other causes; hence, mere hypocellular particles in bone marrow aspiration smears cannot be taken as diagnostic. In two cases, quite a few (around 10%) normocellular particles were seen in the background of predominantly hypocellular particles. This was attributed to aspiration from a 'hot spot', or an area of bone marrow in which hematopoiesis is not affected to its full extent. In such cases of variable cellularity, it was reported to be difficult to describe the overall cellularity of the bone marrow depending upon aspiration smears. Bone marrow biopsy was the tool that gave us the final and accurate details of marrow cellularity and proportions of each individual cellular element. It nullified the bias created by normocellular particles in marrow aspiration smears. All the problems of assessing marrow cellularity arising from blood tap and paucicellular smears were solved by biopsies.

As regard to serum level of IL-8 in this study, There was significant increase between studied groups as P-value was <0.001, also a significant increase between (II & I), (II & III), (II & IV), (I & III), (I & IV) (p<0.001), where in group (I), it ranged between 61.4 – 70.2 (pg/ml) with a mean of 66.175±3.608 in group (II), it ranged between 13.9 – 15.1 (pg/ml) with a mean of 14.569±0.386, in group (III), it ranged between 0.9-2.3 (pg/ml) with a mean of 1.750±0.599 and in group (IV), it ranged between 1.1 – 2.8 (pg/ml) with a mean of 2.000±0.587, but there was non-significant difference between (III & IV) (P = 0.991).

These findings are consistent with those of Tang *et al.* [29], who compared the serum IL-8 levels of 24 AA cases with those of 20 healthy subjects. It was found that IL-8 levels were considerably (P 0.05) greater in AA cases than in healthy controls, with levels in the severe AA group being significantly higher than those in the chronic AA group. Furthermore, Tang *et al.* [29] found elevated levels of IL-8 in the sera of AA patients, with a link between chemokine levels and disease severity; this finding suggests that IL-8 levels may be useful in discriminating between severe and non-severe types of AA.

Moreover, in the present study, a significant negative correlation was found between serum level of IL-8 and Hb level, platelets count, neutrophil count and reticulocyte count as P-value =0.001, &0.002&<0.001) respectively. Also, a significant positive correlation was found between serum level of IL-8 and ESR as P-value <0.001.

As regard group (I), a significant negative correlation was found between serum level of IL-8 and Hb level & platelets count as P-value =0.002 and 0.036, but non-significant correlation was found between it and age, neutrophil count, reticulocyte count, LDH level and ESR. In group (II), there was non-significant correlation was found between serum level of IL-8 and (age, Hb level, platelets count and neutrophil count, reticulocyte count, LDH level and ESR). As P-value =(0.378 &0.067& 0.314 &0.442&0.810&0.181 and 0.178) respectively. As regard group (III), there was statistically non-significant correlation was found between serum level of IL-8 and (age, Hb level, platelets count and neutrophil count, reticulocyte count, LDH level and ESR) as P-value = (0.946 &0.364& 0.886& 0.129 &0.154& 0.329 and 0.648) respectively

At cutoff >2.8, the sensitivity of IL-8 for correlated with AA severity was 80.0%, the specificity was 100.0%, with positive predictive volume is 100.0% and negative predictive volume is 62.5 and accuracy (area under the curve) is 86.8%.

In a study by Jaworska-Wilczyńska *et al.* [30] conducted by in postpericardiotomy syndrome (PPS). The authors reported that a threshold of 21.1 pg/ml of IL-8 was determined to evaluate its prognostic potential. The results showed a sensitivity of 62.5%, a precision of 75%, a positive predictive value of 83%, and a negative predictive value of 50%.

Increased amounts of this cytokine in the blood may lead to activation-induced mortality of adult neutrophils in the periphery because IL-8 is also a powerful activating factor for neutrophils. The exact process by which IL-8 inhibits marrow function is not yet understood [31]. Based on their findings, Broxmeyer *et al.* [32] hypothesised that IL-8 could limit myelopoiesis by preventing the proliferation of myeloid progenitors through a receptor-ligand-mediated process. The activation-induced mortality of peripherally located mature neutrophils may also be triggered by elevated amounts of this cytokine in the circulation.

Limitations: The number of patients included in the present study was small so, further studies of serum level of IL-8 on a large scales is recommended in addition to estimation serum level of IL-6 and IL-17

Conclusions

The level of serum IL-8 is higher in AA patients than in healthy subjects and it is more increase in very severe AA patients (group I) than severe AA patients (group II) than non-severe AA patients (group III), that suggested it may be involved in the mechanisms of AA hence it correlates with the disease progression and there is significant negative correlation was found between serum level of IL-8 and Hb level, platelets count, neutrophil count, reticulocyte count and BM cellularity. Also, a significant positive correlation was found between serum level of IL-8 and ESR.

Conflict of Interest

Not available

Financial Support

Not available

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