Expression of Antinuclear Antibodies and Their Nuclear Pattern in Patients with Acute Leukemia

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Abstract

Background: Antinuclear antibodies are seen in autoimmune diseases and malignancies. These indicators are critical for early cancer diagnosis. The prognosis is affected by ANAs and malignancy.

Objectives: Explore the association between ANA and acute leukemia, and categorize the patterns by indirect immunofluorescence (IIF).

Patients and methods: This cross-sectional study was conducted at Qena University Hospital, Egypt, from November 2021 to May 2022. All previously diagnosed acute leukemia cases that fulfilled inclusion criteria were studied and subjected to complete blood count, serum ANA detection, and identification of the ANA patterns by indirect immunofluorescence (IIF).

Results: 51 patients, 31 (60.78%) females and 20 (39.22%) males, 38 (74.5%) acute lymphoblastic leukemia (ALL) represented, and acute myeloid leukemia (AML) 13 (25.5%). Positive ANA cases were 16 (31.37%). The positive group was significantly younger (16.3 ± 13.11 years) versus the negative group (34.63 ± 19.37 years). Eosinophil percentage was significantly lower in the positive group (0.55 ± 0.92 %) versus the negative group (1.19 ± 1.22 %). Monocyte count was significantly increased in cases with homogeneous cases compared to speckled.

Conclusion: The ANA's diverse IIF expression patterns (homogeneous, nuclear, and speckled patterns) in acute leukemia patients indicate a potential association with immune system alteration and autoantibody production targeting various nuclear components.

Keywords: Autoantibodies; ANA; ELISA; IF.

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Introduction

Immunological tolerance breakdown precipitates the formation of autoantibodies in conditions extending beyond autoimmune disorders, notably malignancies **(Wang et al., 2020).** Antinuclear antibodies (ANAs) represent autoantibodies that exhibit reactivity towards normal human cell nuclear and cytoplasmic constituents **(Vlagea et al., 2018).** Recent investigations have ascertained the presence of ANAs within the bloodstream of patients diagnosed with a variety of tumors **(Gogas et al., 2006),** thereby establishing their utility as serological markers for autoimmune disorders.

Autoantibodies are recognized as participants in the process of carcinogenesis **(Fernández-Madrid et al., 2015),** and ANA titers hold the potential to facilitate the early identification of various malignancies **(Loke et al., 2018).** These autoantibodies exhibit notable sensitivity and specificity in the detection of breast and other malignancies **(Chapman et al., 2007).** Furthermore, the presence of positive ANAs in malignancies exerts an impact on cancer prognosis **(Wu et al., 2017).** Notably, melanoma patients who develop autoantibodies or manifest clinical signs of autoimmunity during interferon alfa-2b therapy experience statistically significant improvements in both relapse-free survival and overall survival **(Gogas et al., 2006).**

In non-small cell lung cancer patients treated with nivolumab or pembrolizumab, preexisting ANAs are associated with therapeutic benefits and immune-related adverse events **(Toi et al., 2019).**

The International Consensus on ANA Patterns (ICAP) has outlined the nuclear patterns of ANAs via indirect immunofluorescence (IIF), based on the specific antigens targeted **(Damoiseaux et al., 2019).** Different ANA patterns have demonstrated correlations with cancer. Homogenous and speckled immunofluorescence patterns in ANAs typically indicate the absence of malignancy, while nuclear patterns in ANAs suggest a potential association with cancer **(Gauderon et al., 2020).** It is essential to note that the presence of anti-centromere antibodies significantly elevates the statistical risk of cancer **(Higuchi et al., 2000).**

Despite their well-documented association with malignancies, the therapeutic relevance of ANAs, particularly their distinct nuclear patterns, within the context of leukemia remains unclear. This cross-sectional study aims to elucidate the relationship between ANAs, specifically their nuclear patterns, and outcomes in patients diagnosed with acute leukemia. Notably, the presence of ANAs exhibiting a nuclear pattern is anticipated to be predictive of a poor prognosis, thereby offering valuable insights into the prognosis of acute leukemia **(Wang et al., 2021).**

This study aims to investigate the prevalence of the ANAs expression and their associated nuclear patterns in acute leukemia patients.

Patients and methods

This cross-sectional study was conducted in the Clinical and Chemical Pathology Department as well as the Internal Medicine Department and Clinic at Qena University Hospital, Egypt. The study included all acute leukemia cases either newly diagnosed or under treatment, during six months from November 2021 to May 2022. Patients who declined to participate in the study were excluded from the research.

Clinical and laboratory assessments involved gathering anthropometric data such as height, and weight, and calculating the Body Mass Index (BMI).

All cases were subjected to the following

Blood sampling: 5 ml of blood was obtained from each participant and divided into 2 tubes: 2 ml of blood in an EDTA tube for a complete blood count (CBC) using a Yumizen H550 analyzer (HORIBA ABX SAS-France) and 3 ml of blood in a plain tube that was left to clot, then centrifuged. Sera were aliquoted in cryotubes and stored at -80°C until ANA analysis.

The ANA IgG antibodies in human serum were determined by ELISA assay with the Quanta Lite® ELISA kit (Cat No. 708750, INOVA Diagnostics, Barcelona, Spain), following the manufacturer's guidelines for ANA detection. The cut-off values were defined as negative < 20 units, moderate positive 20–60 units, and strong positive > 60 units.

The ANA patterns were identified by indirect immunofluorescence (IIF) using fluorescent conjugate (FITC-labeled anti-human IgG) (INOVA Diagnostics Inc., San Diego, CA, USA) Quanta Lite® and NOVA LiteTM assays. Identification of positive and negative results was carried out using an Olympus BX51 fluorescence microscope, with manual interpretation involving careful examination of cell distribution and fluorescence uniformity at varying magnifications.

The study was performed after approval from the Ethics Committee Review Board (IRB) and the Ethical Code: SVU-MED-CCP031-1-21-11-274.

Statistical Analysis

SPSS software (version 24) was used for data analysis. The Shapiro-Wilk test was employed to assess the normality of the distribution of continuous variables. Levene's test was conducted to assess the homogeneity of variances across different groups. Qualitative variables were expressed as frequencies and percentages, compared with the chi-square test. Quantitative measures were shown as means ± standard deviation and compared with the student t-test for normal distributed data, and the Mann-Whitney U test for not normal distributed data. A p-value < 0.05 was deemed statistically significant.

Results

The study involved 51 participants, with a mean age of 22.05 ± 17.41 years, 20 (39.22%) males and 31 (60.78%) females. 38 (74.5%) of the participants had ALL, including 30 (58.82%) ALL1, 7 (13.73%) ALL2, and 1 (1.96%) ALL3]. 13 (25.5%) of the participants had AML, including 2 (3.92%) M1, 3 (5.88%) M2, 1 (1.96%) M3, 2 (3.92%) M4, 3 (5.88%) M5, 1 (1.96%) M6, and 1 (1.96%) M7. All patients received blood transfusions. The ANA diagnostic tests revealed 16 (31.37%) positive for ELISA and IIF, with either homogeneous pattern in 3 (5.88%). nuclear patterns in 3 (5.88%), a speckled pattern in 10 (19.61%), **(Table.1).**

The results of positive ANA by ELISA and IIF examinations patterns showed insignificant differences between ALL and AML groups, (P= 0.18331), **(Table.2).**

Table 1. Characteristics of included subjects

Variables	Value (N = 51)
Age (Years) (Mean ± SD)	22.05 ± 17.41
Sex N (%)	
• **Male**	20 (39.22%)
• **Female**	31 (60.78%)
Type of leukemia N (%)	
• **ALL**	38 (74.5%)
• **ALL1**	30 (58.82%)

• **ALL2**	7 (13.73%)
• **ALL3**	1 (1.96%)
• **AML**	13 (25.5%)
• **M1**	2 (3.92%)
• **M2**	3 (5.88%)
• **M3**	1 (1.96%)
• **M4**	2 (3.92%)
• **M5**	3 (5.88%)
• **M6**	1 (1.96%)
• **M7**	1 (1.96%)
Therapy (N (%))	20 (39.22%)
Blood transfusion N (%)	51 (100%)
Positive ELISA N (%)	16 (31.37%)
Positive for Immunofluorescence N (%)	16 (31.37%)
Pattern N (%)	
• **Homogeneous**	3 (5.88%)
• **Nuclear**	3 (5.88%)
• **Speckled**	10 (19.61%)

Table 2. Characteristics of positive ANA and IIF cases subjects

Variables	Positive ELISA and IF Group (N = 16)
Age (Years)	16.3 ± 13.11
Sex	
· **Male**	15 (42.86%)
· **Female**	20 (57.14%)
Type of leukemia	
· **ALL**	28 (80%)
· **AML**	7 (20%)
Pattern	
· **Speckled**	10 (62.5%)
· **Homogeneous**	3 (18.75%)
· **Nuclear**	3 (18.75%)
CBC	
RBC (10^6/ µL)	2.42 ± 0.79
HGB (g/dl)	6.97 ± 2.13
HCT (%)	21.05 ± 6.19
MCV (fL)	87.98 ± 7.46
MCH (pg)	28.48 ± 2.6
MCHC (g/dL)	32.85 ± 1.6
RDW (%)	17.2 ± 4.27

PLT (x10^3/µL)	35.32 ± 24.7
WBCs	
TLC (x10^3/µL)	32.79 ± 54.36
Neutrophils (x10^3/µL)	23.04 ± 15.14
Lymphocytes (x10^3/µL)	59.25 ± 24.02
Monocytes (x10^3/µL)	14.95 ± 20.93
Eosinophils (x10^3/µL)	0.55 ± 0.92
Basophils (x10^3/µL)	0.68 ± 2.48
Bone Marrow examination	
Promyelocytes (%)	1.57 ± 2.62
Metamyelocytes (%)	0.91 ± 1.25
Myelocytes (%)	0.69 ± 0.68
Band Cells (%)	0.71 ± 1.23
Segmented Neutrophils (%)	7.11 ± 4.5
Lymphocytes (%)	10.86 ± 7.29
Monocytes (%)	2.14 ± 2.24
Eosinophils (%)	1.26 ± 0.89
Basophils (%)	0.6 ± 0.69
Plasma Cells (%)	0.29 ± 0.62
Erythroid Cells (%)	25.63 ± 11.32
Blast Cells (%)	48.34 ± 18.75

The ALL group's mean age was significantly lower (18.36 ±17.12years) than the AML group's (32.85 ± 13.8 years), (p = 0.0082). The TLC in the AML group was significantly higher (74.02 x 10^3/µL) than in the ALL group (30.17 x 10^3/µL), (p = 0.00855). Neutrophils were likewise substantially higher in the AML group (32.38 ± 23.43) compared to the ALL group (20.41 ±10.5), (p = 0.01627). In contrast, the AML group significantly had lower levels of lymphocytes and monocytes than the ALL group, both with (P = 0.0001). Basophils and eosinophils did not show any discernible differences, **(Table.3)**.

The bone marrow examination data showed lower segmented neutrophils in the AML group compared to the ALL group (P= 0.00355), no detectable plasma cells in the AML group, and no significant differences in the erythroid cells, blast cells, or immunofluorescence patterns between the two groups, (**Table.3)**.

The results of positive ANA by ELISA and IIF examinations patterns showed insignificant differences between ALL and AML groups, (P= 0.18331), **(Table.3)**.

Table 3. Leukemia type concerning different parameters

Variables	ALL Group (N = 38)	AML Group (N = 13)	P-Value
Age (Years)	18.36 ± 17.12	32.85 ± 13.8	0.0082*
	9.5 (2-60)	34 (4-60)	
Sex			
· **Male**	16 (42.11%)	4 (30.77%)	0.4699
· **Female**	22 (57.89%)	9 (69.23%)	
CBC			
RBC (10^6/µL)	2.35 ± 0.73	2.64 ± 0.75	0.2337
HGB (g/dl)	6.81 ± 2.01	7.53 ± 2	0.2683
HCT (%)	20.47 ± 5.81	22.74 ± 5.57	0.2257
MCV (fL)	88.52 ± 7.25	85.42 ± 8.26	0.2046
MCH (pg)	29.02 ± 2.78	27.68 ± 3.52	0.1675
MCHC (g/dL)	33.03 ± 1.54	32.84 ± 2.31	0.7446
RDW (%)	16.95 ± 3.84	18.38 ± 3.27	0.2376
PLT (x10^3/µL)	35.72 ± 22.2	43.83 ± 31.46	0.3134
WBCs Data			
TLC (x10^3/µL)	30.17 ± 34.75	74.02 ± 80.04	0.00855*
Neutrophils (x10^3/µL)	20.41 ± 10.5	32.38 ± 23.43	0.01627*
Lymphocytes (x10^3/µL)	67.82 ± 12.74	18.89 ± 12.11	<0.0001*
Monocytes (x10^3/µL)	10.38 ± 8.63	44.58 ± 34.59	<0.0001*
Eosinophils (x10^3/µL)	0.7 ± 1.07	0.9 ± 1.05	0.5664
Basophils (x10^3/µL)	0.29 ± 0.45	1.63 ± 4.01	0.04417*
Bone Marrow Examination			
Promyelocytes (%)	1.21 ± 1.17	2.77 ± 4.55	0.0551
Metamyelocytes (%)	0.87 ± 1.21	0.62 ± 0.65	0.4779
Myelocytes (%)	0.79 ± 0.74	0.69 ± 0.63	0.6744
Band Cells (%)	0.68 ± 1.19	0.85 ± 1.72	0.7083
Segmented Neutrophils (%)	7.71 ± 4.5	5.62 ± 4.72	0.1587
Lymphocytes (%)	10.53 ± 7.06	4.31 ± 3.07	0.00355*
Monocytes (%)	1.97 ± 2.09	1.15 ± 1.77	0.2111
Eosinophils (%)	1.18 ± 0.9	0.69 ± 0.75	0.0822
Basophils (%)	0.55 ± 0.65	0.46 ± 0.78	0.6783

Plasma Cells (%)	0.26 ± 0.6	0	-
Erythroid Cells (%)	24.53 ± 11.01	25.85 ± 14.25	0.7311
Blast Cells (%)	49.82 ± 18.62	57.15 ± 23.12	0.2547
ELISA and indirect immunofluorescence examination			
Positive ELISA	10 (26.32%)	6 (46.15%)	0.18331
Immunofluorescence			
Positive result	10 (26.32%)	6 (46.15%)	0.18331
The pattern of ANA			
• **Homogeneous**	1 (2.63%)	2 (15.38%)	0.09163
• **Nuclear**	2 (5.26%)	1 (7.69%)	0.74798
• **Speckled**	7 (18.42%)	3 (23.08%)	0.71513

*: Significant; RBC: Red Blood Cells; HGB: Hemoglobin; HCT: Hematocrit; MCV: Mean Corpuscular Volume;MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; RDW: Red Cell Distribution Width; PLT: Platelets

The individuals with positive ELISA and IIF results were younger (16.3 ± 13.11 years vs. with negative ELISA and IIF cases 34.63 ± 19.37 years, p = 0.00024), However, the mean TLC was higher in the negative ANA cases (60.07 ± 45.87 x103/µL) compared to positive ANA cases (32.79 ± 54.36 $x10^3$/µL). Moreover, the eosinophils count was significantly higher in ANA negative cases (1.19 ± 1.22 vs. ANA positive cases 0.55 ± 0.92), (p = 0.04247). Conversely, there were no significant differences in numerical blood parameters, except for lymphocytes, and monocytes. The gender distribution and distribution of ALL and AML cases did not differ significantly between the two groups, **(Table.4)**.

Table 4.ANA results from Crithidia luciliae concerning different patients parameters

Variables	Positive ELIZA and IF Group (N = 16)	Negative ELIZA and IF Group (N = 35)	P-Value
Age (Years)	16.3 ± 13.11	34.63 ± 19.37	0.00024*
Sex			
· **Male**	15 (42.86%)	5 (31.25%)	0.4308
· **Female**	20 (57.14%)	11 (68.75%)	
Type of leukemia			
· **ALL**	28 (80%)	10 (62.5%)	0.1833
· **AML**	7 (20%)	6 (37.5%)	
CBC			
RBC (10^6/ µL)	2.42 ± 0.79	2.44 ± 0.62	0.9357
HGB (g/dl)	6.97 ± 2.13	7.04 ± 1.78	0.9132

HCT (%)	21.05 ± 6.19	21.04 ± 4.97	0.9913
MCV (fL)	87.98 ± 7.46	87.18 ± 8	0.7281
MCH (pg)	28.48 ± 2.6	29.1 ± 3.8	0.4994
MCHC (g/dL)	32.85 ± 1.6	33.27 ± 2.06	0.4287
RDW (%)	17.2 ± 4.27	17.58 ± 2.15	0.7408
PLT (x10^3/µL)	35.32 ± 24.7	43.19 ± 24.92	0.2976
WBCs			
TLC (x10^3/µL)	32.79 ± 54.36	60.07 ± 45.87	0.0878
Neutrophils (x10^3/µL)	23.04 ± 15.14	23.81 ± 16.02	0.8687
Lymphocytes (x10^3/µL)	59.25 ± 24.02	46.28 ± 25.52	0.0873
Monocytes (x10^3/µL)	14.95 ± 20.93	28.18 ± 27.83	0.0656
Eosinophils (x10^3/µL)	0.55 ± 0.92	1.19 ± 1.22	0.04247*
Basophils (x10^3/µL)	0.68 ± 2.48	0.51 ± 0.75	0.789
Bone Marrow examination			
Promyelocytes (%)	1.57 ± 2.62	1.69 ± 2.44	0.8814
Metamyelocytes (%)	0.91 ± 1.25	0.56 ± 0.63	0.292
Myelocytes (%)	0.69 ± 0.68	0.94 ± 0.77	0.2435
Band Cells (%)	0.71 ± 1.23	0.75 ± 1.57	0.9301
Segmented Neutrophils (%)	7.11 ± 4.5	7.31 ± 4.96	0.8882
Lymphocytes (%)	10.86 ± 7.29	4.75 ± 2.74	0.0022*
Monocytes (%)	2.14 ± 2.24	0.94 ± 1.12	0.04754*
Eosinophils (%)	1.26 ± 0.89	0.63 ± 0.72	0.01587*
Basophils (%)	0.6 ± 0.69	0.38 ± 0.62	0.2729
Plasma Cells (%)	0.29 ± 0.62	0	0.0736
Erythroid Cells (%)	25.63 ± 11.32	23.19 ± 12.95	0.4979
Blast Cells (%)	48.34 ± 18.75	59 ± 20.91	0.0754

*: Significant; RBC: Red Blood Cells; HGB: Hemoglobin; HCT: Hematocrit; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; RDW: Red Cell Distribution Width; PLT: Platelets

There was no significant difference between different IIF patterns except for blood monocyte count which showed a significant increase in homogenous pattern cases compared with Speckled. **(Table.5).**

Table 5.Immunofluorescence patterns concerning patient data

Variables	Homogeneous (N = 3)	Nuclear (N = 3)	Speckled (N = 10)	P1	P2	P3
Age	**51.33 ± 15.01**	44 ± 9.17	26.8 ± 19.14	0.5101	0.0685	0.1689
Sex						
· **Male**	**1 (33.33%)**	1 (33.33%)	3 (30%)	0.99	0.9214	0.9214
· **Female**	**2 (66.67%)**	2 (66.67%)	7 (70%)			
Type of leukemia						
· **ALL**	**1 (33.33%)**	2 (66.67%)	7 (70%)	0.5185	0.2904	0.9214
· **AML**	**2 (66.67%)**	1 (33.33%)	3 (30%)	0.5185	0.2904	0.9214
CBC						
RBC (10^{12}/L)	**2.32 ± 0.76**	2.52 ± 0.24	2.45 ± 0.7	0.6913	0.7935	0.8697
HGB (g/dl)	**6.97 ± 2.15**	7.7 ± 0.7	6.86 ± 1.99	0.605	0.9364	0.4982
HCT (%)	**20.3 ± 6.06**	22.6 ± 2.27	20.79 ± 5.55	0.5715	0.8983	0.6006
MCV (fL)	**87.93 ± 10.09**	89.63 ± 2.47	86.21 ± 8.92	0.7909	0.78	0.5358
MCH (pg)	**30.3 ± 5.38**	30.6 ± 1.97	28.29 ± 3.85	0.9321	0.4796	0.3486
MCHC (g/dL)	**34.23 ± 2.16**	33.63 ± 1.82	32.87 ± 2.18	0.7317	0.3613	0.595
RDW (%)	**17.2 ± 3.55**	19.13 ± 1.68	17.22 ± 1.82	0.4419	0.9894	0.1336
PLT (x10^3/µL)	**55 ± 29.1**	62.33 ± 34.56	33.9 ± 17.8	0.7926	0.1432	0.0734
WBC						
TLC (x10^3/µL)	**68.16 ± 75.02**	68.87 ± 7.37	55.01 ± 46.55	0.9879	0.7127	0.6279
Neutrophils	**14.6 ± 9.02**	29.07 ± 27.38	25 ± 14.29	0.4338	0.2662	0.7296
Lymphocytes	**22.5 ± 24.11**	51.17 ± 16.17	51.95 ± 25.81	0.1624	0.1073	0.9619
Monocytes	**62.47 ± 33.48**	17.83 ± 16.6	20.99 ± 22.27	0.1074	**0.0269***	0.8264
Eosinophils	**0.13 ± 0.06**	1.67 ± 2.01	1.37 ± 1.05	0.2572	0.0743	0.7318
Basophils	**0.3 ± 0.44**	0.2 ± 0.17	0.67 ± 0.91	0.7306	0.5172	0.4039
Bone marrow differential cell %						
Promyelocytes	2.33 ± 1.53	0.67 ± 0.58	1.8 ± 2.97	0.1518	0.7752	0.5369
Metamyelocytes	1 ± 1	0.67 ± 0.58	0.4 ± 0.52	0.6433	0.1774	0.4591
Myelocytes	1.33 ± 1.15	0.67 ± 0.58	0.9 ± 0.74	0.4217	0.4442	0.6281
Band Cells	0.67 ± 1.15	2 ± 3.46	0.4 ± 0.7	0.5614	0.6232	0.1586
Segmented Neutrophi	9 ± 6.56	5.33 ± 2.52	7.4 ± 5.27	0.417	0.6688	0.534
Lymphocytes	5.33 ± 4.51	4.67 ± 2.08	4.6 ± 2.63	0.8276	0.7228	0.9689
Monocytes	0.67 ± 1.15	0.33 ± 0.58	1.2 ± 1.23	0.6779	0.519	0.2722
Eosinophils	0	0.33 ± 0.58	0.9 ± 0.74	-	-	0.2516
Basophils	0.67±0.58	0.33 ± 0.58	0.3 ± 0.67	0.5185	0.4155	0.9401
Erythroid Cells	20.67±16.26	24.67 ± 13.65	23.5 ± 13.29	0.7605	0.7622	0.8968
Blast Cells	58.33±30.04	60.33 ± 17.56	58.8 ± 21.43	0.9255	0.9762	0.9128

P1 (homogeneous vs. nuclear), P2 (homogeneous vs. speckled), P3 (nuclear vs. speckled); *: Significant; RBC: Red Blood Cells; HGB: Hemoglobin; HCT: Hematocrit; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; RDW: Red Cell Distribution Width; PLT: Platelets.

Discussion

ANA patterns play a role in cancer diagnosis and prognosis. **Gauderon et al. (2020)** reported homogeneous and speckled ANA patterns. The presence of diverse ANA patterns in acute leukemia may reflect distinct immunological responses or the generation of nuclear autoantibodies (**Hossain et al., 2014**).

Identification of (ANA) in leukemia patients is emerging as a crucial aspect of research and diagnosis. The presence of ANA may indicate immune dysregulation, offering insights into autoimmune-related complications in leukemia. This connection could refine diagnostics, prognosis, and therapeutic approaches, enhancing personalized care for individuals with this hematologic malignancy and advancing oncoimmunology (**Vlagea et al., 2018; Bloch, 2020).**

We investigated 51 participants, 20 males (39.22%) and 31 females (60.78%). Three age groups were identified: 2-20 years (27 participants, 52.94%), 20-40 years (16 subjects, 31.37%), and 40-60 years (8 subjects, 15.69%). They had 13 AML (25.5%) and 38 ALL (74.5%). The most common age group in our study was 2-20 years old. AML cases were older than ALL cases, ,—these findings match leukemia epidemiology, suggesting age at diagnosis variety. Children and young adults are more likely to get ALL than older people; hence, the two types of leukemia have a large age gap **(Shallis et al., 2019; Tebbi, 2021).**

Our findings contrasted with those of **Wang et al. (2021),** who evaluated 196 adult leukemia patients, with a mean age of 45.6 years, of all cases 108 were males and 88 were females, comprising CML cases 9 (13.2%), CLL cases 4 (5.9%), and ALL cases 19 (27.9%). Involving (55.1%) males and (44.9%) females. They reported positive ANA and IIF in 68 patients (34.7%).

In our study, there were no significant variations in sex distribution or age between ANA-positive and ANA-negative groups this was in agreement with **(Wang et al., 2021)**.

In our Study, 16 (31.3%) of acute leukemia cases (ALL and AML) had positive ANA, with insignificant difference between groups, (p= 0.18331). Positive ANA reactions showed various nuclear patterns, including Homogeneous (3 participants, 5.88%), Nuclear (3 subjects, 5.88%), and Speckled Pattern (10 subjects, 19.61%).

Wang et al. (2021) identified Negative (65.3%), Nuclear Speckled (10.7%), Cytoplasmic Speckled (6.1%), and Nuclear (12.8%) ANA patterns in acute leukemia patients. Notably, our study revealed a dominant Speckled Pattern (62.5%) in ANA positive cases, which contrasts with **Wang et al. (2021)** higher incidence of Nuclear Pattern (12.8%). This discrepancy may stem from demographic or leukemia subtype variations.

The presence of ANAs in a significant proportion of acute leukemia patients suggests the occurrence of an autoimmune response influenced by the malignancy's impact on the immune system. The targeting of nuclear components by ANAs underscores the intricate immune reactions observed in leukemia patients (**Hossain et al., 2014**).

The prognostic value of ANAs is validated by studies in chronic lymphocytic leukemia (CLL). **Sun et al. (2019)** established a link between positive ANA results and poorer outcomes in CLL, suggesting that interactions involving the B cell receptor influence disease progression and survival.

In contrast, **Blaes et al. (2000)** identified ANAs as a protective immune response in

non-small cell lung cancer patients. Targeting specific nuclear antigens was associated with prolonged disease-free survival in lung cancer patients, as reported by **Fernández-Madrid (1999).**

Cabrera et al. (2016) found that nuclear ANA patterns in systemic lupus erythematosus (SLE) complicate interpretation. The presence of various nuclear patterns in acute leukemia raises questions about disease mechanisms and potential associations with autoimmune comorbidities.

The presence of ANAs introduces immunological considerations into the diagnosis of acute leukemia, focusing on the identification and management of leukemia cells. In favorable cases, nuclear patterns may provide insights into autoimmune processes and their influence on prognosis and treatment, thereby enhancing disease management (**Basu et al., 2015**).

Wang et al. (2021) reported a stronger association between ANA nuclear patterns and older patients (≥60 years), supporting the link between age and ANA positivity in acute leukemia. Our data demonstrated a positive correlation between age and ANA-positivity, implying that older individuals are more likely to exhibit ANA reactivity. This reinforces **Wang et al. (2021)** observations that ANA patterns exhibit age-related shifts in immune responses. We revealed an association between age and specific patterns, such as Homogeneous and Nuclear, suggesting age-related modifications in immune responses that warrant further exploration.

Age exhibited a strong correlation with ANA patterns and positivity, while sex and leukemia type did not. This implies that age may play a more significant role in determining ANA presence compared to other demographic factors. The molecular and cellular distinctions among leukemia subtypes may influence the relationship between ANAs and leukemia type **(Bolouri et al., 2018; Hennrich et al., 2018).**

Wang et al. (2021) also established a connection between targeted therapy (Tyrosine Kinase Inhibitors - TKI) and chemotherapy, which increased the prevalence of the nuclear pattern in ANA patterns compared to allogeneic hematopoietic stem cell transplantation (allo-HSCT). This interaction between medication and ANA expression may alter disease outcomes and therapeutic responses. Additionally, the ANA nuclear pattern is linked to reduced survival time, suggesting that ANAs could serve as prognostic indicators in acute leukemia.

Conclusion

The ANA IIF expression patterns in acute leukemia patients indicate a potential association with autoimmune processes. The diverse immunofluorescence patterns (homogeneous, nuclear, and speckled patterns) may reflect distinct immune reactions or specific autoantibodies targeting different nuclear components.

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