Serum estrogen and its soluble receptor levels in Egyptian patients with acute leukemia: case-control study

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Abstract

Background: Acute leukemias are malignant neoplastic diseases that arise from either lymphoid [ALL] or myeloid [AML] cell lines that are distinguished by the proliferation of BM non-functional immature cells and subsequently released into the bloodstream. ALL is prevalent malignancy in young, while AML in older. Diagnosis is usually routinely performed through peripheral blood count and smear then confirmed by BM aspirate. It is remarkable to notice that leukemia can be manifested at high, low, and even at normal leucocyte count. While treatment results have improved steadily over the last decades in younger and adults, limited changes have been in survival among subjects of age > 60 years. Aim of the work is to measure the serum estrogen [E2] and its soluble receptor [ER] levels in acute leukemia patients and extrapolate its possible clinical significance. This study included 40 [20 females and 20 males] healthy volunteers clinically free from any disease, 40 [20 females and 20 males] AML patients, and 40 [20 females and 20 males] ALL. To all subjects, serum E2 and its soluble ER level were investigated by ELISA.

Results: Serum E2 [pg/ml] level was lower in AML and ALL female and male patients groups than control group. Serum ER [ng/ml] level was lower in AML and ALL female and male patients groups than control group.

Conclusion: Estimation of serum E2 and its soluble ER level is of edifying diagnostic value. Determination of serum E2 and its soluble ER level in AML and ALL patients is of value in deciding treatment therapeutic target protocol.

Keywords: Estrogen, Estrogen receptor, Acute leukemia

Background

Acute leukemia is a bone marrow [BM] malignant disease in which the normal hematopoietic marrow cells replaced by an early proliferative myeloid or lymphoid precursors [1–3]. Acute leukemias are characterized upon their differentiation into lymphoid or myeloid lineages. In acute lymphoblastic leukemia (ALL), the abnormal proliferation in immature lymphocytes or lymphoid progenitor cells [4].

Two major types of ALL are known B-ALL and T-ALL. AML implicates the myeloid series from which

neutrophils, eosinophils, basophils, monocytes, and megakaryocytes are derived [5, 6]. Each leukemia type has its own morphological cytochemical and immunological differences, different prognostic markers, and lines of treatment. Prognostic factors could predict treatment outcome in acute leukemia patients, either complete remission after chemotherapy or disease resistance to conventional protocols [7]. A single prognostic factor cannot reliably predict prognosis, but it must be correlated with all available information [8].

Estrogen negatively regulates BM cells proliferation, which turn into progenitors of myeloid and lymphoid. Moreover, significant bone marrow hematopoiesis alteration is present in estrogen insufficiency and in ER knockout mice [9]. Estrogen functions through alpha

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ER-α and beta ER-β receptor [10]. Estrogen receptor gene is present on chromosome 6 long arm, which is often altered in hematopoietic neoplasms [11, 12]. In healthy controls, ER-α is unmethylated compared to acute leukemia patients; hence, it can act as an epigenetic biomarker of leukemia [12]. A previous study found that estrogen suppresses the stem cells differentiation into myeloid and lymphoid. In addition, serum estrogen [E2] has a negative effect which might be by ER-β on immune system [13]. In vitro studies on leukemia have revealed various cytotoxic impact of clomiphen, a well-known ER antagonist in breast cancer patients [14, 15].

Aim of the work
Measure the serum E2 and its soluble ER levels in acute leukemia patients and extrapolate its possible clinical significance as diagnostic markers.

Methods
Subjects submitted work were grouped into the following: *group I*, 40 healthy [20 females and 20 males] clinically free from any disease as control group and their age was 41.30 ± 3.46 years and were chosen from the stuff members and their relatives of university hematology unit; *group II*, 40 AML patients [20 females and 20 males]; and *group III*, 40 ALL patients [20 females and 20 males]. From all participants, informed consent was taken [recruited from MRI and Faculty of Medicine, Alexandria University Hematological unit] in this study.

Exclusion criteria
Subjects with former hematological disorders [myeloproliferative disorders, myelodysplastic syndromes, multiple myeloma, and lymphoproliferative disorders] prior received radio- or chemotherapy for mass neoplasm

Regiments of treatment
45 mg/m^2 Daunorubicin for 3 days and 100 mg/m^2 × 2/day cytosine arabinoside for a week made up the protocol for AML patients.

1.4 mg/m^2 Vincristine days 1, 8, 15, and 22; 1 mg/kg/day Prednisolone × 28 days; and 25 mg/m^2 Doxorubicin days 1, 2, and 3 made up the protocol for ALL patients.

At the protocol end and restoration BM cellularity, aspiration of BM was taken. Less than 5% BM blasts were considered complete remission and 2nd induction cycle was taken by those who did not reach complete remission.

The subsequent investigations were performed for all subjects: complete history and clinical examination enrollment, complete blood picture [16], and some liver functions; AST, ALT, albumin, and some kidney functions; and urea, creatinine [17–20], bone marrow examination [21], flow cytometry to differentiate AML from

ALL [16], and determination of serum soluble ER level by ELISA [22], also E2 level by ELISA [23].

Statistical analysis
Data assessment by SPSS program V20.0, the K-S test was used to check normality. Results were represented as mean [range; min and max], median, and standard error. Significance was considered at 5% level. F-test [ANOVA] and LSD test were used for quantitative variables [parametric]; Kruskal-Wallis test and Dunn's test Spearman coefficient for quantitative variables [non-parametric]; and ROC curve for diagnostic power of test measured by assessing area under curve also a comparison of performance between two tests.

Results
Serum ER level [ng/ml] in AML and ALL male/female patients and control group
The results showed that the level of serum ER [ng/ml] in AML and ALL male patients was significantly lower than in male control group [$P_1 \leq 0.001$, $P_2 = 0.002$]. While levels of ER in both groups of patients were insignificant about same range [$P_3 = 0.915$] (Table 1).

The results showed that the level of serum ER [ng/ml] in AML and ALL female patients was significantly lower than in female control group [$P_1 = 0.012$, $P_2 = 0.003$]. While, levels of ER in both groups of patients were insignificant about same range [$P_3 = 0.629$] (Table 1).

Serum E2 level [pg/ml] in AML and ALL male/female patients and control group
The results showed that the level of serum E2 [pg/ml] in AML and ALL male patients was significantly lower than in male control group [$P_1 = 0.001$, $P_2 = 0.005$], while levels of E2 in both groups of patients were insignificant about same range [$P_3 = 0.920$] (Table 1).

The results showed that the level of serum E2 [pg/ml] in AML and ALL female patients was significantly lower than in female control group [$P_1 = 0.009$, $P_2 = 0.041$]. While, levels of E2 in both groups of patients were insignificant about same range [$P_3 = 0.059$] (Table 1).

Serum ER level [ng/ml] in AML [M4+M5] and AML [other subtypes] male/female patients
The results showed that level of serum ER [ng/ml] in AML [M4 + M5] male patients was insignificantly lower than in AML [other subtypes] patients (Table 2).

The results showed that level of serum ER [ng/ml] in AML [M4 + M5] female patients was insignificantly higher than in AML [other subtypes] patients (Table 2).

Table 1 Serum ER level (ng/ml) and E2 level (pg/ml) in AML and ALL male/female patients and control group

	Control (*n* = 20)	AML (*n* = 20)	ALL (*n* = 20)
ER (ng/ml) in male			
Range	1.17–9.27	0.36–14.11	0.30–2.47
Mean ± SE	5.67 ± 0.81	1.91 ± 0.75	1.24 ± 0.26
Median	5.08	0.75	0.98
H(p)	15.485*(< 0.001*)		
p_1	< 0.001*		
p_2	0.002*		
p_3	0.915		
ER (ng/ml) in female			
Range	0.72–7.75	0.51–11.30	0.24–1.67
Mean ± SE	5.29 ± 0.81	2.37 ± 1.09	0.94 ± 0.11
Median	5.66	0.96	0.94
H(p)	10.278*(0.006*)		
p_1	0.012*		
p_2	0.003*		
p_3	0.629		
E2 (pg/ml) in male			
Range	10.80–44.10	2.70–69.80	3.20–33.40
Mean ± SE	29.96 ± 3.12	15.07 ± 3.90	12.52 ± 3.64
Median	31.80	8.61	10.10
H(p)	11.713*(0.003*)		
p_1	0.001*		
p_2	0.005*		
p_3	0.920		
E2 (pg/mL) in female			
Range	2.90–154.0	2.50–16.70	2.80–20.80
Mean ± SE	51.99 ± 17.48	5.70 ± 1.41	8.80 ± 1.60
Median	35.10	4.05	7.70
H(p)	9.456*(0.009*)		
p_1	0.009*		
p_2	0.041*		
p_3	0.059		

H, *p*, *H* and *p* values for Kruskal-Wallis test. Pairwise comparison bet. each of the 2 groups was done using post hoc test (Dunn's for multiple comparisons test). p_1, *p* value for comparing between control group and AML group; p_2, *p* value for comparing between control group and ALL group; and p_3, *p* value for comparing between AML group and ALL group
*Statistically significant at *p* ≤ 0.05

Serum E2 level [pg/ml] in AML [M4+M5] and AML [other subtypes] male/female patients

The results showed that level of serum E2 [pg/ml] in AML [M4 + M5] male patients was insignificantly lower than in AML [other subtypes] patients (Table 2).

The results showed that level of serum E2 [pg/ml] in AML [M4 + M5] female patients was insignificantly lower than in AML [other subtypes] patients (Table 2).

Liver function parameters in AML and ALL patients groups and control subjects

The results showed that the mean value of AST levels [U/l] in ALL patients is significantly higher than in control group [P_2 = 0.007]. Moreover Table 3 showed that ALT levels [U/l] in AML and ALL patients were significantly higher than in control group [P_1 ≤ 0.001, P_2 ≤ 0.001]. While, serum albumin concentration [mg/dl] in AML and ALL patients were significantly lower than in control group [P_1 = 0.024, P_2 = 0.007] (Table 3).

Kidney function parameters in AML and ALL patients and control group

The results showed that in AML and ALL patients, the mean values of serum urea concentration [mg/dl] [P_1 ≤ 0.001, P_2 ≤ 0.001] and serum creatinine concentration [mg/dl] [P_1 = 0.034, P_2 ≤ 0.001] were significantly higher than in control group. Also, the mean value of serum creatinine concentration [mg/dl] in ALL patients group was significantly higher than in those with AML [P_3 = 0.008] (Table 4).

Mean values of WBC count [× 10³/µl], PLT count [× 10³/µl], and hemoglobin conc. [g/dl] in AML and ALL patients and control group

The results showed that WBCs count mean value in AML and ALL patients were higher than in control group [P_1 = 0.014, P_2 ≤ 0.001], while Hb conc. and PLT count in both patients groups were lower than in control group [P_1 ≤ 0.001, P_2 ≤ 0.001] (Table 5).

Correlation of serum ER and E2 levels with different biochemical and hematological parameters in AML and ALL male/female group

As presented in Table 6, level of ER [ng/ml] in serum of AML male patients showed a significant positive correlation with E2 [pg/ml] [r_s = 0.472, p = 0.044] and Hb concentration [g/dl] [r_s = 0.472, p = 0.048] and was negatively correlated with WBCs count [× 10³/µl] [r_s = − 0.489, p = 0.040] and age [years] [r_s = − 0.729, P = 0.001] (Table 6).

Our results showed that serum E2 [pg/ml] was positively significantly correlated with ER [ng/ml] [r_s = 0.636, p = 0.048] and Hb concentration [g/dl] [r_s = 0.754, p = 0.012] in AML female patients group. Also, it was noticed that level of serum ER [ng/ml] of ALL female patients showed a negative significant correlation with Blast cells [r_s = − 0.665, p = 0.036], while level of serum E2 [pg/ml] of ALL female patients showed a negative significant correlation with albumin [mg/dl] [r_s = − 0.661, p = 0.038] (Table 6).

Table 2 Serum ER level (ng/ml) and E2 level (pg/ml) in AML (M4+M5) and AML (other subtypes) male/female patients

	AML (M4 + M5) (n = 10)	AML (others subtypes) (n = 10)
ER (ng/ml) in male		
Range	0.36–3.58	0.36–14.11
Mean ± SE	1.42 ± 0.39	2.52 ± 1.66
Median		
U(p)	38.500(0.894)	
ER (ng/ml) in female		
Range	0.59–11.30	0.51–5.40
Mean ± SE	2.85 ± 2.11	1.90 ± 0.89
Median		
U(p)	11.00(0.754)	
E2 (pg/ml) in male		
Range	3.20–25.90	2.70–69.80
Mean ± SE	11.63 ± 2.52	19.38 ± 8.25
Median	8.61	8.95
U(p)	39.500(0.965)	
E2 (pg/mL) in female		
Range	2.60–6.14	2.50–16.70
Mean ± SE.	3.96 ± 0.71	7.43 ± 2.64
Median	3.0	4.30
U(p)	9.00(0.465)	

U, Mann-Whitney test; P, P value for comparing the two studied groups

Table 3 Liver functions in AML and ALL patients and control group

Liver function	Control (n = 40)	AML (n = 40)	ALL (n = 40)	Test of sig.	p
SGOT (U/l)					
Range	21.0–35.0	9.0–100.0	15.0–100.0	$H = 7.444$*	0.024*
Mean ± SE	28.20 ± 1.10	39.04 ± 4.31	48.21 ± 5.73		
Median	28.50	37.50	50.00		
Sig. bet. grps	$p_1 = 0.101$, $p_2 = 0.007$*, $p_3 = 0.188$				
SGPT(U/l)					
Range	10.0–19.0	5.0–130.0	30.0–110.0	$H = 33.282$*	< 0.001*
Mean ± SE	15.30 ± 0.83	46.07 ± 6.03	53.34 ± 4.73		
Median	17.50	39.00	49.00		
Sig. bet. grps	$p_1 < 0.001$*, $p_2 < 0.001$*, $p_3 = 0.102$				
Alb (mg/dl)					
Range	3.60–4.70	2.40–4.90	2.50–4.90	$F = 5.632$*	0.006*
Mean ± SE.	4.31 ± 0.07	3.83 ± 0.13	3.64 ± 0.19		
Median	4.40	3.80	3.60		
Sig. bet. grps	$p_1 = 0.024$*, $p_2 = 0.007$*, $p_3 = 0.485$				

H, p, H and p values for Kruskal-Wallis test. Pairwise comparison bet. each of the 2 groups was done using post hoc test (Dunn's for multiple comparisons test). F, p, F and p values for ANOVA test. Pairwise comparison bet. each of the 2 groups was done using post hoc test (LSD). p_1, p value for comparing between control group and AML group; p_2, p value for comparing between control group and ALL group; and p_3, p value for comparing between AML group and ALL group
*Statistically significant at $p \leq 0.05$

Table 4 Kidney functions in AML and ALL patients and control group

Renal function	Control ($n = 40$)	AML ($n = 40$)	ALL ($n = 40$)	H	p
Urea (mg/dl)					
Range	10.50–36.50	9.0–200.0	17.0–160.0	23.784*	< 0.001*
Mean ± SE	24.02 ± 1.52	46.07 ± 6.75	56.99 ± 8.72		
Median	23.80	40.00	40.00		
Sig. bet. grps	$p_1 < 0.001*, p_2 < 0.001*, p_3 = 0.444$				
Cr (mg/dl)					
Range	0.50 – 1.10	0.50 – 5.0	0.40 – 4.0	19.388*	< 0.001*
Mean ± SE	0.71 ± 0.04	1.11 ± 0.17	1.95 ± 0.27		
Median	0.67	0.80	1.30		
Sig. bet. grps	$p_1=0.034*, p_2<0.001*, p_3=0.008*$				

H, p, *H* and *p* values for Kruskal-Wallis test. Pairwise comparison bet. each of the 2 groups was done using post hoc test (Dunn's for multiple comparisons test). p_1, *p* value for comparing between control group and AML group; p_2, *p* value for comparing between control group and ALL group; and p_3, *p* value for comparing between AML group and ALL group
*Statistically significant at $p \leq 0.05$

Comparison between the values of serum ER and E2 as diagnostic marker for AML and ALL male/female patients groups

The ROC curve plot was applied for assessment the diagnostic values of serum ER [ng/ml] and E2 [pg/ml] based on the AUC. Serum ER showed significant AUC [0.926], *P* [< 0.001] with sensitivity and specificity [96.30% and 90.0%, respectively], and cut-off value [≤ 3.58 ng/ml]. Serum E2 [pg/ml] showed significant AUC [0.870] [*P* = 0.001], with sensitivity and specificity [85.19% and 80.0%, respectively] (Table 7 and Fig. 1A).

The ROC curve plot was applied for assessment the diagnostic values of serum ER [ng/ml] and E2 [pg/ml] based on the AUC. Serum ER showed significant AUC [0.880] [P = 0.001] with sensitivity and specificity [90.0% and 90.0%, respectively], and cut-off value [≤ 1.67 ng/ml]. Serum E2 [pg/ml] showed significant AUC [0.808] [P = 0.005] with sensitivity and specificity [100% and 60.0%, respectively] (Table 7 and Fig. 1B).

Discussion

The role of estrogen receptor has been well established in cancer breast. Several studies have explored its role in

Table 5 Hematological parameters in AML and ALL patients and control group

	Control ($n = 40$)	AML ($n = 40$)	ALL ($n = 40$)	Test of sig.	p
WBCs ($\times 10^3$/μl)					
Range	3.20–10.0	0.54–130.0	1.0–170.0	H = 16.452*	<0.001*
Mean ± SE	6.01 ± 0.45	35.88 ± 7.0	56.76 ± 10.25		
Median	5.60	19.00	51.50		
Sig. bet. grps	$p_1 = 0.014*, p_2 < 0.001*, p_3 = 0.054$				
PLTs ($\times 10^3$/μl)					
Range	136.0–300.0	6.0–296.0	7.0–334.0	H = 35.230*	< 0.001*
Mean ± SE	229.10 ± 12.04	65.11 ± 10.66	66.52 ± 16.12		
Median	228.50	50.00	54.00		
Sig. bet. grps	$p_1 < 0.001*, p_2 < 0.001*, p_3 = 0.945$				
Hb (g/dl)					
Range	9.80–14.0	5.50–12.20	4.50–12.70	F = 26.230*	< 0.001*
Mean ± SE	11.90 ± 0.29	8.76 ± 0.28	8.52 ± 0.50		
Median	12.25	9.00	8.50		
Sig. bet. grps	$p_1 < 0.001*, p_2 < 0.001*, p_3 = 0.929$				

H, p: H and p values for Kruskal-Wallis test. Pairwise comparison bet. each of the 2 groups was done using post hoc test (Dunn's for multiple comparisons test). *F, p*, F and p values for ANOVA test. Pairwise comparison bet. each of the 2 groups was done using post hoc test (LSD). p_1, *p* value for comparing between control group and AML group; p_2, *p* value for comparing between control group and ALL group; and p_3, p value for comparing between AML group and ALL group
*Statistically significant at $p \leq 0.05$

Table 6 Correlation of ER and E2 with different biochemical and hematological parameters in male/female groups

| Male	AML (*n* = 20)				ALL (*n* = 20)			
	ER		E2		ER		E2	
	r$_s$	*p*	*r*$_s$	*p*	*r*$_s$	*p*	*r*$_s$	*p*
ER (ng/ml)	-	-	0.472*	0.044*	-	-	-0.317	0.406
E2 (pg/ml)	0.472*	0.044*	-	-	− 0.317	0.406	-	-
Age (years)	− 0.729*	0.001*	− 0.335	0.174	− 0.343	0.366	0.075	0.847
Hb (g/dl)	0.472*	0.048*	0.184	0.465	− 0.167	0.667	− 0.100	0.797
WBCs (× 10^3/µl)	− 0.489*	0.040*	− 0.322	0.192	− 0.183	0.637	0.233	0.546
PLTs (×10^3/µl)	− 0.028	0.911	0.289	0.245	− 0.059	0.881	− 0.293	0.444
SGOT (U/l)	0.001	0.998	0.028	0.912	− 0.151	0.698	0.160	0.682
SGPT (U/l)	0.313	0.206	0.094	0.710	0.034	0.931	0.322	0.398
UR (mg/dl)	− 0.080	0.751	− 0.181	0.471	0.339	0.372	0.051	0.897
Cr (mg/dl)	− 0.015	0.953	− 0.284	0.253	− 0.211	0.586	0.158	0.685
Blast cells	− 0.013	0.961	0.032	0.899	0.042	0.914	− 0.498	0.173
Alb (mg/dl)	0.160	0.526	0.330	0.182	− 0.351	0.354	− 0.251	0.515
Female	AML (*n* = 20)				ALL (*n* = 20)			
	ER		E2		ER		E2	
	r$_s$	*p*	*r*$_s$	*p*	*r*$_s$	*p*	*r*$_s$	*p*
ER (ng/ml)	-	-	0.636*	0.048*	-	-	0.457	0.184
E2 (pg/ml)	0.636*	0.048*	-	-	0.457	0.184	-	-
Age (years)	0.358	0.310	0.176	0.627	0.236	0.511	0.156	0.668
Hb (g/dl)	0.353	0.318	0.754*	0.012*	0.363	0.302	0.227	0.529
WBCs (× 10^3/µl)	− 0.389	0.266	− 0.395	0.258	0.000	1.000	− 0.085	0.815
PLTs (× 10^3/µl)	0.134	0.712	0.561	0.092	− 0.068	0.853	− 0.109	0.763
SGOT (U/l)	− 0.343	0.333	− 0.037	0.920	0.117	0.747	0.140	0.699
SGPT (U/l)	− 0.366	0.298	0.098	0.789	− 0.209	0.562	− 0.158	0.663
UR (mg/dl)	− 0.109	0.763	0.103	0.776	− 0.050	0.892	− 0.263	0.463
Cr (mg/dl)	0.306	0.390	0.128	0.724	− 0.588	0.074	− 0.543	0.105
Blast cells	0.603	0.065	0.382	0.277	− 0.665*	0.036*	− 0.534	0.112
Alb (mg/dl)	− 0.219	0.544	0.067	0.854	− 0.526	0.118	− 0.661*	0.038*

rs Spearman coefficient
*Statistically significant at *p* ≤ 0.05

Table 7 ROC curves analysis of serum ER (ng/ml) and E2 (pg/ml) in AML and ALL male/female patients groups

	AUC	Asymptomatic significance	Cut-off	Sensitivity	Specificity
Male					
ER	0.926*	< 0.001*	≤ 3.58	96.30	90.0
E2	0.870*	0.001*	≤ 25.9	85.19	80.0
Female					
ER	0.880*	0.001*	≤ 1.67	90.0	90.0
E2	0.808*	0.007*	≤ 23.45	100.0	60.0
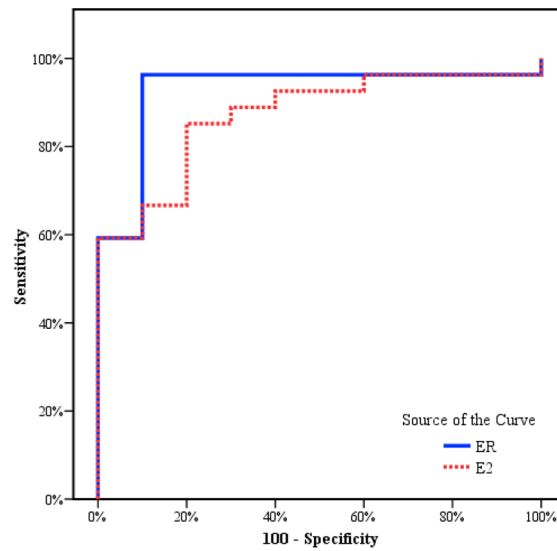
(a): Serum ER (ng/ml) and E2 (pg/ml) in AML and ALL male patients groups ROC curve
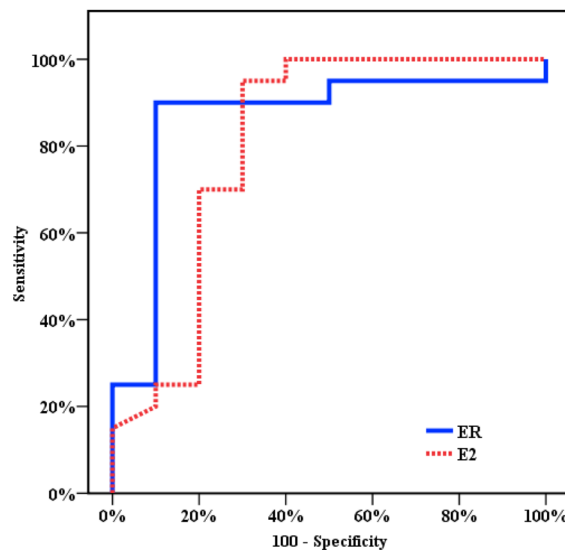
(b): Serum ER (ng/ml) and E2 (pg/ml) in AML and ALL female patients groups ROC curve

Fig. 1 A Serum ER (ng/ml) and E2 (pg/ml) in AML and ALL male patients groups ROC curve. B Serum ER (ng/ml) and E2 (pg/ml) in AML and ALL female patients groups ROC curve

different cancers, notably mass tumors such [24–27] for which anti-estrogens were tried in an attempt to cure these malignancies. Yet the estrogen and its soluble estrogen receptor clinical significance in acute leukemia patients have not been investigated. Our work hypothesizes that determining the E2 and soluble ER levels could provide valuable information in treating acute leukemia patients.

The non-steroidal anti-estrogens [AE] are a vast class of artificial compounds that are derived from triphenylethylene as tamoxifen. They are estrogen antagonists whose cellular effects are not merely by estrogenic blockade [28].

In cells of breast cancer, tamoxifen induces in vitro TGF-B1 and phospholipases expression activates cellular. It can arrest the BC cell cycle in G phase [29]. It has anti-angiogenic action is not interceded via ER [30].

Anti-estrogens [AE] exert oxidative stress, influencing calcium signaling [31], and conduct the action of variant receptors away of ER. Moreover, AEs induce apoptosis via caspase activity [32, 33] and antagonize drug resistance [28].

Hayon et al. [28] investigated the ER distinctive effects of anti-estrogens on ALL cell lines. Their findings revealed that anti-estrogens have growth inhibitory effects and by means of apoptosis and opposing of drug impedance.

These effects were confirmed when AEs were but together with other cytotoxic drugs. They added that cell cycle progression block may occur in leukemic cells ER deficient.

In the current work, we assessed the soluble ER in patients with acute leukemia. The mean soluble ER was lower in patients compared to the control in both AML and ALL patients. The low serum soluble ER in patients could reflect a low ER expression on leukemic cells.

Our findings especially in ALL patients whose ER levels were lower than AML agrees with, Hayon et al. [28] who reported that lymphoblastic cells do not express estrogen receptors and the anti-estrogens role in their study which involved apoptosis induction was ER independent. They found that nafoxidine, another anti-estrogen, proved to be more potent than tamoxifen or clomiphene.

The difference between the three anti-estrogens could be due to affinity binding difference to anti-estrogen binding sites.

We could attribute the low level of ER in acute leukemias to possible methylation and consequently gene silencing. This has been revealed in previous studies which demonstrated that ERs expression could be controlled by genetic and epigenetic mechanisms in human cancers [34, 35].

Yao et al. [12], studied CPG promoter methylation of estrogen receptors in leukemia. They used RT-PCR and MSP-PCR in leukemia cell lines and direct DNA sequencing. They reported that only ER α was specifically methylated and inactivated nearly in all acute leukemia patients while no methylation in control group which agrees with our findings where serum ER was elevated in the control upon comparing with the leukemic patients. This highlights that silencing of the gene expressing ER by methylation can be important in pathogenesis of leukemia or it is partially depending on the carcinogenic insult that induced the neoplastic disease [12].

Cytosine methylation inactivates genes participate in neoplasia or tumor suppressor genes. The degree of hyper methylation is due to DNA methyltransferase upregulation.

In the current work, the range of serum level of soluble serum ER was variable and large and this could be explained by that not all AML subgroups do express the ER equally. We could postulate that the AML patient's different behavior is due to either the different leukemic subtypes or to the state of their cholesterol metabolism. Yom-Tov et al. [15] and de Medina et al. [36] stated that anti-estrogen can function as a ligand for anti-estrogen binding microsomal site, generating cell death through cholesterol metabolism regulation.

In addition, the degree of methylation in AML patients all subtypes is not the same. Toyota et al. [37] studied the aberrant methylation profile in AML. They deduced that hyper methylation of some genes associated with reduced levels of their expression and they found that age inversely correlated with the number of methylated genes. This agrees with our study, as we reported an inverse significant correlation between the mean serum ER level and age, yet it was only for male patients, we could not establish this correlation in females whether AML or ALL.

This elucidate relation between methylation and age is significant in older patients retain little genes methylated and that of AML biology in elderly is totally unlike AML young patients. This agrees with Qingli et al. who found a negative association between age and ER this reflects that AML biology in adults is different from that in elderly or the different triggering factors that led to AML.

As regards the serum ER levels was lower in M4 and M5 male patients compared to other subtypes in males yet the difference was not statistically significant.

This could be interpreted by the small sample size or different cell of origin in M4 and M5. Yet, in female patients, the mean level of serum ER in M4 and M5 patients was higher than the other subtypes. This indicates that the different levels of serum ER with specific subtypes highlight different methylation levels.

Moreover, the serum ER lower level in ALL compared to AML patients could be attributed to the occurrence of hyper ethylating phenotypes in ALL than in AML.

This agrees with Toyota et al. [37] whose preliminary data suggested the occurrence of hyper methylation phenotypes in ALL reflecting different gene expression profiles, implying the presence of specific carcinogenic insults such as radiation exposure or previous cytotoxic chemotherapeutic drugs.

In the current work, significant elevated ALT and AST levels were reported in patients versus the control. This was notable in ALL compared to AML patients reflecting that the leukemic impact is more prominent on the liver in ALL patients. The same findings were reported in renal function tests which were more elevated in ALL than AML patients.

Soluble ER can be a biological marker of leukemia. ER α A the isoform in comparison to other isoforms of ER was specifically and highly methylated in leukemic patients and was no methylation in controls [12]. Li et al. [38] added that the different levels of methylated ER reflect different exposure to carcinogenic insults.

In the current work, the estrogen level was elevated in the control than the patients and that was statistically

significant both in AML and ALL males and females patients.

In the current work, the mean serum albumin was positively correlated with ER in AML male patients, and this was not the same in AML or ALL female patients.

In the current work, AML male patients had a lower ER level than females. This agrees with previous studies who found a higher ER methylation and subsequent lower level of ER among males. These findings reflect an association between ER levels with sex [37, 38].

The significant decline in serum of ER levels and E2 concentration in male and female patients groups with acute leukemias compared to their corresponding normal controls propose the capability of applying any one of these variables in acute leukemia diagnosis to distinguish the patients with acute leukemias from normal controls. This led us to compare the diagnostic power of these indices to decide which of decisive diagnostic value. This comparability also concerned with identification of the precision specificity and sensitivity for each parameter and their corresponding cut-off value. This comparability was achieved through ROC curve plotting in such a way that the greatest plot below the ROC curves consistent with superior diagnostic test.

Serum ER either in male or female patients showed the greater area below the curve [0.926 and 0.880, respectively] followed by E2 [0.870 and 0.808, respectively].

Cut-off values, specificity, and sensitivity for diagnostic power male and female patients with acute leukemia were 3.58 ng/ml, 90%, 96.3% and 1.67 ng/ml, 90%, and 90% for ER and 25.9 pg/ml, 80%, 85.19% and 23.45 pg/ml, 60%, and 100% for E2, respectively.

These results indicate that serum ER is superior to serum E2 for diagnosis of male and female acute leukemia patients. Despite serum ER and E2 having been detected in acute leukemia patients, to our knowledge, this is the first work to compare diagnostic significance for serum ER with those of serum E2 with estimation of the precise cut-off value, specificity, and sensitivity of each parameter in acute leukemia patients.

Conclusion

1. The soluble ER in both males and AML and ALL females was lower significantly than the group of control.
2. Level serum E2 was lower in patients whether males or females than the control group.
3. ER level significantly positively correlated with hemoglobin concentration in AML male patients.
4. Total leukocytes count inversely correlated with ER level in AML male patients group.

5. Serum ER was significantly negative correlated with blast percent in ALL female patients group.

Recommendations

1. E2 and its soluble ER should be involved in the diagnostic workup to acute leukemia and especially the AML.
2. ER expression and methylation level should be studied especially in AML patients, in an attempt to target this receptor by anti-estrogens.

Declarations

Ethics approval and consent to participate
The research methodology in the present work was approved by research ethical committee of Alexandria University (0304933, Alexandria, Egypt) and written informed consent was obtained from each participant enrolled in the study prior to sample collection. Experimental procedures and sampling followed the international and national regulations in accordance with the Declaration of Helsinki.

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.

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