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ORIGINAL ARTICLE

Serum Estrogen and its Soluble Receptor Levels in Egyptian Patients with Chronic Myeloid Leukemia: A Case–Control Study

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Abstract Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder. CML cells contain a BCR-ABL gene, not typically found in normal cells that produce a protein (BCR-ABL) causing CML cells to proliferate. CML occurs in three phases: chronic, accelerated and blast crisis. Disease staging is primarily based on percent of blasts in the blood and bone marrow. Most cases of CML are diagnosed in chronic phase (CP). The major objective in CML clinical management is to prevent progression from chronic to accelerated and blast crisis phases. While earlier treatments, such as cytoreductive chemo- and interferon therapies increased overall survival rates among patients, the advent of tyrosine-kinase inhibitors (TKIs) have changed the CML treatment landscape. Despite the widespread use of these therapies, there have also been associated side effects that could potentially affect its use. Also it is necessary to avoid all deaths and complications related to the treatment, by limiting as much as possible the side-effects of the treatment while ensuring the compliance of the patients. The aim of this work was to measure the serum estrogen and its soluble receptor levels in patients with chronic myeloid leukemia in order to extrapolate their possible clinical significance. The present study included 40 (20 males and 20 females) healthy volunteers clinically free from any disease, 40 (20 males and 20 females) patients of newly diagnosed CML. Blood samples were collected from all subjects and the level of serum estrogen (E2) and serum soluble estrogen receptor (ER) were measured by enzyme linked immunosorbent assay (ELISA). The level of serum E2 (pg/ml) in both male and female patients groups with CML was significantly higher than in control group. The level of serum ER (ng/ml) in both male and female patients groups with CML was significantly lower than in control group. Estimating the serum level of E2 and soluble ER is of informative diagnostic value. Estimation serum level of E2 and soluble ER in patients with CML is of value in deciding use of antiestrogen as therapeutic target in treatment protocol.

Keywords Chronic myeloid leukemia · Estrogen · Estrogen receptor

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Introduction

Chronic myeloid leukemia (CML) is a pluripotent hematopoeitic stem cell neoplasm characterized by the BCR-ABL1 fusion gene, derived from a balanced translocation

between the long arms of chromosomes 9 and 22 t (9;22)(q34;q11.2) known as the Philadelphia chromosome. It is present in 95% of CML cases [1].

The BCR-ABL fusion gene gives rise to hybrid transcripts, translated into an oncoprotein which has a constitutive active tyrosine kinase activity. It has the capacity to phosphorylate a number of cytoplasmic substrates with other activities of the chimeric protein leading to alterations in proliferation, differentiation, adhesion and survival [2, 3].

The leukemic clone has a tendency to acquire additional oncogenic mutations over time. This clonal evolution is associated with progression to the accelerated and the terminal blastic phases of the disease or resistance to tyrosine kinase inhibitors [4].

Identification of the association of Philadelphia chromosome and tyrosine kinase resulted in the successful development of Imatinib mesylate which is a protein inhibitor of the BCR-ABL tyrosine kinase as well as other subclass III receptor tyrosine kinases including c-kit and platelet derived growth receptor (PDGF-R) [5].

Although imatinib has dramatically reduced the yearly risk of CML progression, it does not eradicate the disease and in most patients, leukemic stem cells persist [6, 7]. Resistance to tyrosine kinase inhibitor causes structural cleavage that closes the ATP-binding factor of BCR-ABL that the drug cannot enter and inhibit the kinase domain [8]. Clinical trials have formally demonstrated that in CML patients treated with TKI who achieved and maintained deep molecular responses could discontinue their treatment after several years without facing overt signs of disease relapse in approximately 50% of the cases. In patients with a molecular relapse, prompt re-introduction of TKI therapy was able to rapidly restore deep molecular responses. The concept of a lifelong therapy with TKI has thus been challenged and treatment-free remission (TFR) strategies will soon integrate clinical practice, providing that safe recommendations will be established [9].

Sokal score is a multivariate prognostic model that can help predict shorter survival and make decisions regarding use of aggressive therapy [10].

It has been reported that estrogen deficiency and in estrogen receptor knockout mice, there is significant alteration in bone marrow hematopoiesis [11]. Estrogen exerts its effect through two distinct receptors, estrogen receptor (alpha ER-α and beta ER-β). Estrogen receptor alpha has been detected in non-hematopoietic cells in bone marrow and in B lymphocyte precursors in mouse [12]. Estrogen can still partially decrease the percentage of c-kit + cells in the absence of ER -α,suggesting that estrogen receptors are mediator of estrogenic control of c-kit + cells [13]. Studies of ovariectomized mice have shown that ovarian hormone deficiency promotes the expansion of a pool of bone marrow derived progenitors that differentiate into myeloid and lymphoid cells. Normal peripheral monocytes originating from bone marrow myeloid cells, predominantly express ER-β proteins and undergo apoptosis after estrogen exposure [14].

Aim of the Work

The aim of this work was to measure the serum estrogen and its soluble receptor levels in patients with chronic myeloid leukemia in order to extrapolate their possible clinical significance.

Subjects and Methods

Individuals submitted to this study were divided into two groups.

Group I

Involved 40 apparently healthy volunteers (20 males and 20 females) clinically free from any disease (control group), their mean age was 45.90 ± 6.19 years and were chosen from the stuff members of MRI, Alexandria University and clinical research center, Faculty of Medicine, Alexandria University and their relatives.

Group II

Involved 40 patients of newly diagnosed CML (20 males and 20 females).They were of matched age and sex as the control group and were recruited from Hematological department, MRI, Alexandria University and clinical research center, Faculty of Medicine, Alexandria University. An informed consent was taken from all contributors in this study (10RG#10RG0008812; Alexandria, Egypt).

Patients with CML newly diagnosed in chronic phase were orally treated with Glivec. The dose was 400 ml once or twice daily. Patients with CML that were resistant to Glivec, were orally treated with TASIGNA (300–400 ml / day). Both Glivec and TASIGNA act as tyrosine kinase inhibitor.

To all subjects the following investigations were done:1- Full history recording, 2- Thorough clinical examination 3- Complete blood picture [15], 4- Determination of Sokal scoring [10], 5- Estimation of estrogen level by ELISA [16] and 6- Estimation of soluble estrogen receptor level by ELISA [17].

Statistical Analysis

Statistical analysis was carried out using SPSS statistics software version 20. Quantitative data were tested for normality using Kolmogorov–Smirnov test. Abnormally distributed data was given as range (minimum–maximum). Non-parametric statistical tests of significance were applied; Mann–Whitney test was used to compare two independent groups. All applied statistical tests of significance were two-tailed.

Results

Estrogen E2 (Pg/ml) and Estrogen Receptor ER (ng/ml) levels in Normal Male Control Subjects and Male Patients Group with Chronic Myeloid Leukemia CML

The statistical analysis of these results revealed that the concentration of E2 (Pg/ml) in male CML patients group was significantly higher than in control group ($P_1 = 0.022^*$). While the concentration of ER (ng/ml) in CML male patients group was significantly lower than in male control group ($P_2 = 0.001^*$), Table 1.

Estrogen E2 (Pg/ml) and Estrogen Receptor ER (ng/ml) Levels In Female Control Group and Female Patients Group with Chronic Myeloid Leukemia CML

The statistical analysis of these results revealed that the concentration of E2 in (Pg/ml) in CML female patients group was significantly higher than in normal female control group ($P_1 = 0.023^*$). While the concentration of ER in (ng/ml) in CML female patients group was significantly lower than in control group ($P_2 = < 0.001^{**}$), Table 2.

Sokal Score in CML Male and Female Patients Group

As presented in Table 3, Sokal score in male patients group ranged from 0.69 to 2.09 with a mean value1.03 ± 0.09. Sokal score in female patients group ranged from 0.76 to 2.17 with a mean value 1.23 ± 0.09.

Estrogen E2 (Pg/ml) and Estrogen Receptor ER (ng/ml) levels in Male Patients Group According to Sokal Score

The statistical analyses of these results revealed that E2 level in CML male patients at low, intermediate and high risk were nearly within the same range and showed insignificant difference. In addition ER level in CML male patients at low and high risk were nearly within the same range and significantly lower than in CML male Patients at intermediate risk, Table 4.

Estrogen E2 (Pg/ml) and Estrogen Receptor ER (ng/ml) levels in CML Female Patients Group According to Sokal Score

The statistical analysis of these results revealed that E2 level in CML female patients group at intermediate and high risk were nearly within the same range and insignificantly lower than in CML female patients group at low

Table 1 Statistical analyses of Estrogen E2 (Pg/ml) and Estrogen receptor ER (ng/ml) levels in male control group and CML male patients group

	Control (n = 20)	Cases (n = 20)
E2(Pg/ml)		
Range	8.80 – 27.30	10.90 – 46.20
Mean ± SE	17.86 ± 1.99	26.72 ± 2.33
P1	0.022*	
ER (ng/ml)		
Range	1.44 – 15.50	0.87 – 8.20
Mean ± SE	9.60 ± 1.46	3.21 ± 0.52
P2	0.001*	

P1: Values of E2 compared with control group. P2: Values of ER compared with control

Significant was considered at $p \leq 0.05$

Table 2 Statistical analyses of Estrogen E2 (Pg/ml) and Estrogen receptor ER (ng/ml) levels in female control group and female patients group with chronic myeloid leukemia CML

	Control (n = 20)	Cases (n = 20)
E2(Pg/ml)		
Range	8.24–58.90	10.5–253.0
Mean ± SE	18.40 ± 4.57	95.46 ± 27.61
P1	0.023*	
ER (ng/ml)		
Range	4.14–17.50	0.56–7.80
Mean ± SE	12.03 ± 1.37	3.07 ± 0.53
P2	< 0.001*	

P1: Values of E2 compared with control group. P2: Values of ER compared with control

Significant was considered at $p \leq 0.05$

Table 3 Sokal score in CML male and female patients group

Sokal (male)	
Range	0.69–2.09
Mean ± SE	1.03 ± 0.09
Sokal (female)	
Range	0.76–2.17
Mean ± SE	1.23 ± 0.09

risk, similarly ER level in CML female patients group at intermediate and high risk were nearly within the same range and insignificantly lower than in CML female patients group at low risk, Table 5.

Absolute Lymphocytes (count/µl), Monocytes (count/µl) and lymphocytes/monocytes Ratio in Normal Control Subjects and Patients with CML

The statistical analyses of these results revealed the mean value of absolute Monocytes count in patients group with CML were significantly higher than in control group (P2 < 0.001*). The mean value of absolute lymphocytes count in patients groups with CML was significantly lower than in control group (P1 = 0.001*). Also the mean value of ALC/AMC ratio in patients group with CML was significantly lower than in control group, Table 6.

ALC/AMC in Patients Group According to Sokal Score

The statistical analyses of these results revealed that the mean value of ALC/AMC ratio in patients group either at intermediate or at high risk was significantly lower than in patients group at low risk, also ALC/AMC in patients group at high risk was significantly lower than in patients group at intermediate risk Table 7.

Correlation between Sokal Score with ALC/AMC Ratio in Patients Group

As presented in Table 8, ALC/AMC in patients group has a significant negative correlation with Sokal score.

Correlation between Philadelphia Chromosome % with ALC/AMC Ratio in Patients Group

As presented in Table 9, ALC/AMC in patients group has a significant negative correlation with Philadelphia chromosome %.

Table 4 Statistical analyses of Estrogen E2 (Pg/ml) and Estrogen receptor ER (ng/ml) in male patients group according to Sokal score

	Sokal score		
	< 0.8 Low risk	0.8 – 1.2 intermediate risk	> 1.2 High risk
Estrogen (pg/ml)	(n = 8)	(n = 7)	(n = 5)
Range	16.20 – 45.90	10.90 – 46.20	15.10 – 34.20
Mean ± SE	26.34 ± 3.63	27.61 ± 5.12	25.98 ± 3.07
p	0.942		
p_1	0.949		
p_2	0.570		
p_3	0.685		
ER	(n = 8)	(n = 7)	(n = 5)
Range	0.87 – 5.61	1.50 – 8.20	1.05 – 2.20
Mean ± SE	2.66 ± 0.72	5.01 ± 0.91	1.57 ± 0.18
^{H}p	0.043*		
p_1	0.030*		
p_2	0.818		
p_3	0.032*		

P: P value for **Kruskal Wallis test** for comparing E2 between the three categories, Sig.bet.grps was done using Post Hoc Test (Dunn's multiple comparisons test).^{H}p: ^{H}p value for **Kruskal Wallis test** for comparing ER between the three categories, Sig.bet.grps was done using Post Hoc Test (Dunn's multiple comparisons test)

p_1: p value comparing between < 0.8 and 0.8 – 1.2 p_2: p value comparing between < 0.8 and > 1.2

p_3: p value comparing between 0.8 – 1.2 and > 1.2 *: Statistically significant at $p \leq 0.05$

Table 5 Statistical analyses of Estrogen E2 (Pg/ml) and Estrogen receptor ER (ng/ml) levels in female patients group according to Sokal score

	Sokal score		
	< 0.8 low risk	0.8 – 1.2 intermediate risk	> 1.2 High risk
Estrogen (pg/ml)	(n = 7)	(n = 7)	(n = 6)
Range	30.90 – 253.0	10.50 – 227.0	47.20 – 151.0
Mean ± SE	141.95 ± 111.05	77.32 ± 51.25	90.35 ± 23.62
p	0.683		
p_1	0.355		
p_2	1.000		
p_3	0.564		
ER	(n = 5)	(n = 6)	(n = 9)
Range	1.90 – 7.80	0.56 – 5.02	0.57 – 7.15
Mean ± SE	4.31 ± 1.45	2.43 ± 0.82	2.94 ± 0.78
^{H}p	0.174		
p_1	0.088		
p_2	0.189		
p_3	0.346		

P: P value for **Kruskal Wallis test** for comparing E2 between the three categories, Sig.bet.grps was done using Post Hoc Test (Dunn's multiple comparisons test).^{H}p: ^{H}p value for **Kruskal Wallis test** for comparing ER between the three categories, Sig.bet.grps was done using Post Hoc Test (Dunn's multiple comparisons test)

p_1: p value comparing between < 0.8 and 0.8 – 1.2 p_2: p value comparing between < 0.8 and > 1.2

p_3: p value comparing between 0.8 – 1.2 and > 1.2 *: Statistically significant at $p \leq 0.05$

Table 6 Statistical analyses of Absolute Lymphocytes (count/μl), Monocytes (count/μl) and lymphocytes/monocytes ratio in normal control subjects and patients with chronic myeloid leukemia CML

	Control(n = 40)	Cases(n = 40)
Absolute Lymphocytes		
Range	2256.0 – 4950.0	83.20 – 13,318.0
Mean ± SE	3123.25 ± 161.07	2729.85 ± 418.32
P1	0.001*	
Absolute Monocytes		
Range	75.0 – 360.0	78.0 – 2481.0
Mean ± SE	189.30 ± 20.34	498.74 ± 84.62
P2	0.001*	
ALC/AMC ratio		
Range	8.0 – 43.05	0.43 – 38.0
Mean ± SE	20.81 ± 2.62	9.36 ± 1.45
P3	< 0.001*	

P1: Values of absolute lymphocytes compared with control group

P2: Values of absolute monocytes compared with control group

P3: Values of absolute ALC/AMC ratio compared with control group

*: Significant was considered at $p \leq 0.05$

Table 7 Statistical analysis of ALC/AMC in patients group according to Sokal score

ALC/AMC ratio	Sokal		
	< 0.8 (n = 12)	0.8 – 1.2 (n = 13)	> 1.2 (n = 15)
Range	3.20 – 38.0	0.43 – 38.0	1.13 – 11.0
Mean ± SE	13.15 ± 2.64	8.99 ± 2.84	4.43 ± 0.80
^{H}p	0.005*		
p_1	0.021*		
p_2	0.002*		
p_3	0.465		

^{H}p: ^{H}p value for **Kruskal Wallis test** for comparing ER between the three categories, Sig. bet. grps was done using Post Hoc Test (Dunn's multiple comparisons test)

p_1: p value comparing between < 0.8 and 0.8 – 1.2

p_2: p value comparing between < 0.8 and > 1.2

p_3: p value comparing between 0.8 – 1.2 and > 1.2

*: Statistically significant at $p \leq 0.05$

Discussion

Chronic myeloid leukemia occurs in 15- 20% of adult leukemia and less than 10% of CML patients are below 20 years. The disease runs a chronic phase to be accelerated phase, end by blast crisis. The chronic phase is characterized by high leukocyte count, less than 5% blasts in the peripheral blood, increased basophils and increased or normal platelets counts [18].

In the present study, we observed that males were affected by the disease more than females, this agrees with

Table 8 Correlation between Sokal score with Absolute lymph/mono ratio in patients group (n = 40)

	Sokal score	
	r_s	p
ALC/AMC ratio	− 0.488*	0.002*

rs: Spearman coefficient

*: Statistically significant at $p \leq 0.05$

Table 9 Correlation between Ph. Chromosome % with ALC/AMC ratio in patients group (n = 40)

	Ph. Chromosome %	
	r_s	p
ALC/AMC ratio	− 0.333*	0.038*

rs: Spearman coefficient

*: Statistically significant at $p \leq 0.05$

previous studies. The mean age of the patients was 45.67 ± 9.37 years which agree with Tobassum et al. [19].

Before initiating the treatment, patients are assessed by Sokal score to be stratified into risk profiles. Sokal score criteria are age, spleen size, number of platelets and blast % in peripheral blood. A score below 0.8 indicates low risk, score of 0.8–1.2 represents intermediate risk, while 1.2 or higher score indicates high risk [20].

In the present study, Sokal score varied from 0.69–2.09 with a mean of 1.03 ± 0.4 in males and 0.76 ± 2.17with a mean of 1.23 ± 0.41 in females these values reflect that most of our patients were in the chronic phase.

Before the discovery of tyrosine kinase inhibitors (TKIs) treatment of CML had a poor outcome. Imatinib (Glivec) is the first generation TKI and nilotinib (Tasigna) is one of the 2nd generation TKIs. Their action is mainly by blocking the BCR-ABL tyrosine kinase in their pathways of proliferation. However the only curative therapy of CML is by allogeneic bone marrow transplantation. After initiation of treatment, patients are monitored by quantitative real time PCR for BCR-ABL transcripts on peripheral blood or bone marrow samples. The response to treatment entails hematologic response with normalization of the peripheral blood counts and major cytogenetic response which is assessed at major months intervals [21].

Loss of initial response to first generation TKI might be related to multiple molecular events which make the disease progress from chronic phase to accelerated phase or blast phase due to imatinib resistance [22–24]. It has been reported that cancer stem cells exist which are resistant to therapy, leading to disease progression. These quiescent BCR-ABL positive hematopoietic stem cells constitute around 0.5% of CD34 + cell population and refractory to therapeutic agents and are responsible for persistent residual disease [22]. On the other hand studies have suggested that the resistance of these stem cells to therapy result from BCR-ABL kinase domain mutations that are present at the start or appear during treatment [23, 24].

The chimeric protein has various sizes due to heterogeneity of BCR-ABL rearrangement, importing different clinical behavior as well as response to tyrosine kinase inhibitors [25]. In addition, expression of two or more transcripts has been reported by Yghmaie et al. [25, 26]

In the present study, patients were started on imatinib those who failed to respond to the drug or did not reach molecular remission were shifted to second generation TKI (Tasigna). We could relate the weak response to imatinib to the possible presence of BCR—ABL variant transcripts which were not detected by the conventional PCR. In agreement with our results Prejzner et al. [27] reported on the relationship of the BCR gene breakpoint and the type of BCR/ABL transcript to clinical course, prognosis and survival in patients with CML. These various transcripts are detected by multiplex reverse transcription polymerase chain reaction. More ever Deb et al. [28] studied the correlation of transcript variants with presenting features, risk score and response to imatinib mesylate. Most patients with b2a2 presented with higher Sokal risk score, achieved complete hematologic response at 3 months.

Balatzenko et al. [29] studied the influence of BCR-ABL transcripts on multidrug resistance gene (MDR1) as a possible cause of resistance to imatinib, an intense development of strategies aiming at targeting the stem cell signaling pathways have been advocated [5].

In present study, we estimated estrogen and soluble estrogen receptor in patients with CML in attempt to disclose their relation to sokal score.

In present study, the mean level of soluble ER was statistically significant low in patients groups (males and females) compared to their control groups. This could be explained by lower expression of the receptor on leukemic cells compared to the control. These results are corroborated with Issa et al. [30] who found that estrogen receptor CPG Island is methylated in most hematopoietic neoplasm. The ER gene, which has growth and metastasis suppressor activity in different cells, is inactivated by promoter methylation in ER negative tumor and in hematopoietic neoplasm. [30] The methylation of ER gene is associated with very low even absent ER expression. In addition, this methylation could be a major step in the subsequent production of hematopoietic neoplasms. Hence, it could be a useful molecular marker for monitoring the clinical state of these diseases. Furthermore the ER gene is located on the

long arm of chromosome 6 which is often altered most hematopoietic neoplasms, whether of lymphoid or myeloid origin.

To the best of our knowledge, our study is the first to relate ER to Sokal scoring. In the present study, a statistically significant relation was found between Sokal risk and ER ($H = 6.931$, $p = 0.043$), notably in male patients, the highest ER level was found in those with intermediate risk. In females patients the lowest ER level was found in those with intermediate risk.

On looking to the estrogen level according to Sokal score in our patients; male patients had E2 level nearly within the same range in the different Sokal categories, while in females a marked discrepancy was found in the different Sokal stages. This could be explained by the fluctuation of estrogen levels with menstrual cycle in females, on one hand whether these females are pre or post-menopausal on the other hand.

In our study, the mean level of E2 was significantly high in patients groups (male and female) than their control groups; Yom-Tov et al. [31, 32] conducted a phase II on the use of anti-estrogen as a novel modality for the treatment of acute myeloid leukemia. We could suggest the use of anti-estrogen in treating CML as adjuvant therapy. MandelKar and Kong [33] postulated that the anti-tumor activity of anti-estrogen is ER- independent and is mediated via apoptosis.

In the present study, we observed that male and female patients with high estrogen levels had lower values of ER. This could be explained by lower ER occupancy i.e. more estrogen molecules are circulating in the blood.

In the present study the absolute lymphocyte /monocyte ratio was statistically significantly low in all patients compared to the control ($p < 0.001$). Both lymphocytes and monocytes are biomarkers of immune response and tumor microenvironment. [34] Wilox et al. [35] stated that their score predicts survival and identifies high risk patients with diffuse large B cell lymphoma.

In the present study a statistically negative correlation was found between ALC/AMC ratio and molecular response assessed by PCR (-0.333, $P = 0.038$) this makes it a biomarker of response to therapy. Our results are justified by Yan-Li et al. [36], who stated that this score is independent prognostic marker as lymphocytes included natural killer cells (NK) and mediate antibody-dependent cell mediated cytotoxicity. Patients with higher ALC/AMC ratio fared better than those with a lower ratio.

A striking observation in our study was that the ALC/ AMC ratio negatively correlated with Sokal score. This further confirms that a lower ALC/ AMC ratio and higher Sokal score reflects amore aggressiveness disease status.

Conclusion

From the present study it could be concluded that;

1. The ER is significantly low in patients with CML while E2 is significantly high in CML patients.
2. The ALC/ AMC ratio negatively and statistically correlated with Philadelphia chromosome and Sokal score.

Recommendations

1. Patients with CML should have their ER determined at diagnosis and at molecular monitoring.
2. Anti-estrogen could be tried as novel modality of therapy in patients with CML.
3. The ALC/AMC ratio is a valuable tool in the diagnostic work up and monitoring of CML patients.
4. Further study is needed to disclose any relationship between and ALC/AMC ratio and response to therapy as well as molecular monitoring.

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Availability of Data and Material Data and materials are available upon request.

Declarations

Conflict of interest The authors confirm that they have no competing interests.

Consent for Publication The Authors grant the Publisher permission to publish this work.

Ethics Approval and Consent to Participate The authors give thanks to all the study participants. Written consent to inclusion was obtained from all participants and they were informed of the study. The study was conducted after being authorized by the Medical Ethics Committee of Alexandria Faculty of Medicine.

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