DISCUSSION

The physiology of angiotensin II continues to be a major field of investigation. Recently reported mechanisms suggest that it has growth promoting factor and cytokine like properties in addition to its vasoconstrictor actions.[(169)]

Some of the pathophysiological effects of Ang II may be mediated through activation of the transcription factor nuclear factor KB (NF KB) which participates in a variety of inflammatory responses as well as neoplasia.[(169)]

In addition to ACE dependent pathways of Ang II formation, non ACE pathways have been demonstrated, namely chymase which is a chymotypsin-like serine protease which may represent an improtant mechanism for the conversion of Ang I to Ang II in tissues and in vasculature. In this regard, mast cell chymase immunostaining could identify acute myeloid leukemia.[(181)]

The Ang II T1R has recently been identified, and its mechanisms of action continue to be elaborated.

On the other hand, Ang II T1R mediated over-production of reactive oxygen species (ROS) has potent growth-promoting actions by exerting positive feedback effects that amplifies its signaling in leucocytes and monocytes. Agonist-induced activation of the Ang II T1R Participates in promotion of tumor progression and metastasis though its growth promoting and proangiogenic Ang II T1R.[(182)]

In the present study, the median serum level of ACE was significantly higher in leukemic patients before therapy compared to the control. In the meantime, the level significantly declined post chemotherapy compared to the pre-treatment levels, yet it did not reach the control level in some patients. This reflects the leukemogenic effect of this enzyme especially in patients with AML, this also proved the positive correlation between serum level of ACE and blast percent denoting that these leukemic blasts are incriminated in its production.

Moreover, an inter-individual variability in the serum level of the enzyme was observed in leukemic patients which could be attributed to the possible interplay of ACE gene polymorphism. This is in agreement with Angela et al[(183)] who reported that ACE insertion\ deletion (I\D) polymorphism plays important role in breast cancer risk and disease-free survival in Caucasin postmenopausal women, the D\D carries having an increased risk of developing breast cancer.

The ACE gene is located in chromosome 17 q 23 and has many polymorphisms. The most commonly studied is a 287 bp insertion\deletion polymorphism in intron 16 that accounts for 50% of the variability in circulating ACE.[(184,185)]

Moreover, several studies have shown that Ang II acts as a growth factor in normal and breast cancer cells through phospholipase C activation.[(186)] The deletion polymorphism of ACE gene leads to increased serum levels.[(187)] Alternatively, other as yet unidentified gene loci in linkage disequilibrium with the ART1 1166 variant may account for the different serum levels of the enzyme.

In the present study, we observed that patients with the maximal level of ACE before therapy did not reach a level nearing with the control, but the level remained higher than the control reflecting a state of failure of induction chemotherapy in reducing the burden of leukemic cells. This denotes a more aggressive leukemic blasts possibly bearing the $CD34^+$ immunophenotype and necessitating a more aggressive induction chemotherapy protocol.

Our finding confirms the study of Jokubaitis et al [(188)] who reported that ACE (CD143) marks hemopoietic cell with hemopoietic stem cell characteristics in adult hemopoietic tissues.

The present study is also supported by the report of Aksu et al[(189)] who demonstrated that AML cells overexpress ACE (CD 143), an observation that explains, in part, the relative refractoriness of leukemic cells to the inhibitory effects of chemotherapeutic drugs.[(189)] Their group hyposized the presence of a local bone marrow rennin-angiotensin system affecting physiological and neoplastic blood cell formation. The local RAS has been defined as an autocrine-paracrine system within the hematopoietic lineage and marrow stromal cells[(190)] that may be involved in neoplastic hematopoiesis and leukemogenesis.[(191- 193)]

In the present study, a positive correlation was found between ACE levels and bone marrow blasts, a finding which is congruent with Aksu et al[(189)]. Moreover, several authors reported that renin mRNA was found in leukemic blast cells.[(191, 192)]

Our results were as well explained by Abali et al [(191)] who stated that ACE degrades a tetra-peptide called ACSDKP (goralatide), a negative hematopoietic regulator, hence while peripheral blood ACE levels increase, blast percentages in the bone marrow accumulate and migrate to the circulation. Therefore, ACE hyper function may lead to the enhanced goralatide metabolism, which in turn lowers its level in the bone marrow micro-environment, abolishing the anti-proliferative effect of the peptide on the hematopoietic cells and leukemic blasts.[(192)]

Leukemogenesis is a multistep and multifactorial process which includes activation of oncogenic mutations in signal transduction pathways, conferring a proliferative survival advantage on leukemic cells over normal hematopoietic profanities. Angiogenesis which is neovascularization is one of these crucial pathways in the biology of acute leukemia. In addition to conversion of Ang I to Ang II, ACE breaks down bradykinin (BK) into inactive peptides. Several lines of evidence underline the putative role of BK in the modulation of angiogenesis, hence BK activates angiogenesis[(194)] independent of VEGF pathway.

Similarly, it has been reported that AngII activates the mitogen-activated protein kinase pathway (MAPK), which has a mitogenic effect.[(189)]

On the other hand, recent studies have shown that Ang II and Ang (1-7) also have regulatory effect on tissue regeneration, cellular proliferation and growth factor release.[(195, 196)] In vivo studies have shown that Ang II and Ang (1-7) peptides increased hematopoietic recovery after myelosuppression on multiple blood cell lineages.[(197, 198)] This could explain the post chemotherapy raised level of ACE despite being lower than pre-treatment level. In our study, the enhanced enzyme activity could be beneficial in terms of bone marrow regeneration after the nadir induced by induction chemotherapy. This key component in the rennin-angiotensin system, ACE, in the normal human hemangioblast, supports the critical nature of this enzyme in hematopoietic development.(199)

In support of this finding, Shen et al (199) working on ACE knockout mice, stated that these mice have hematologic developmental defects. They added that an animal lacking all ACE is very different from a wild type animal and can be modeled as representing an extreme complicated phenotype with cardiovascular, reproductive, hematologic and developmental defects.

Another possibility is that post chemotherapy production of ACE might be enhanced by cells other than leukemic blasts. These cells are namely monocytes and macrophages. In this context, Shen et al(199) forwarded substantial evidence that tumor resistance of ACE Knokout mice exhibits a different immune response as compared to wild type animals. Their data include the finding that their knockout mice develop greater numbers of $CD8^+$ cytotoxic T lymphocytes directed at tumor antigens. In addition, the cytokine levels in these mice are different from those in wild type animals.

Similarly, macrophages express Ang II T1R and release angiogenic cytokines, including VEGF, which promote angiogenesis.(200) In this regard, it has been reported that pharmacological blockade of Ang II T1R also reduced tumour angiogenesis, growth and metastasis.(201, 202)

Moreover, it has been reported that mechanisms of Ang IIT1R activation, such as receptor transactivation of tyrosine kinase receptors and stimulation of ROS production suggest that Ang II has growth factor and cytokine-like properties. Receptor transactivation may be defined as that process whereby ligand stimulation of one receptor leads to activation of another distinct receptor.

In the present study, a significant decline in serum levels of Ang IIT1R in patients after therapy, approximately reaching the control level was observed. This could be explained by the decrease in blast percentage by effective chemotherapy at one hand, and by the possibility that the soluble form of the receptor formed a ligand to the remaining receptors expressed on remaining blasts or leukocytes. Hence, they block the receptors leading to abrogation of the signal transduction pathways and dampening of their activation.

In the current study we examined the diagnostic significance of ACE activity (U/L) and Ang IIT1R concentration (U/L) by ROC curve. The curve showed that both parameters were excellent diagnostic markers of acute leukemia as was indicated by asymptomatic significance and very high area under the curve (100%, 100% respectively). The optimum cut off value of ACE activity (U/L) and Ang IIT1R concentration (U/L) were with a corresponding sensitivity 100% and specificity 100% for both parameters.

For the first time, we could define a prognostic value for estimating the levels of ACE activity (U/L) and Ang IIT1R concentration (U/L) in patients with acute leukemia. The Kaplan-Meier disease free survival curve specified a cut off level for ACE < or > than 50.25 U/L with statistical significance ($p < 0.00$). This was true for Ang IIT1R as a cut off value of below or above 386 U/L could discriminate between a higher mean disease free survival of 24 months compared to 14 months in patients with higher level above the cut off point and the difference was statistically significant ($p < 0.001$).

In the present study, a lower serum potassium was observed in patients with acute leukemia, whether ALL or AML, yet AML patients had even lower levels in AML reflecting hypokalemia. We attributed this decrease in serum potassium to a paraneoplastic syndrome or disturbed electrolytes in these patients.

In accordance to our findings, Wulf et al[(203)] reported on paraneoplastic hypokalemia in AML. They stated that it was due to enhanced rennin activity in AML blast cells.

As regards serum albumin in level, it was significantly decreased in patients with both types of acute leukemia denoting liver dysfunction due to leukemic infiltration. Another possibility is that patients with acute leukemia are cachectic and suffer from a negative protein balance, especially with the presence of fever and loss of weight.

In agreement with our results, a low serum albumin is considered as a poor prognostic marker in AML.[(204)]

Regarding serum sodium level, the present study exhibited a significant decrease in serum sodium levels in patients with acute leukemias and the control. This could be ascribed to paraneoplastic production of antidiuretic hormone, a syndrome known as "syndrome of inappropriate antidiuretic hormone" release by the leukemic blasts. This syndrome is sometimes aggravated by chemotherapeutic drugs, especially those used in treating ALL.

The novelty of this study is that it evaluates the impact of induction chemotherapy on serum ACE and soluble Ang II T1R in patients with acute leukemia. Other studies were in-vitro studies.

Hence our study indicates a causal relationship between RAS and acute leukemia imparting that manipulating the rennin-angiotensin pathways and targeting its receptors could be exploited as adjuvant therapy in these patients.

SUMMARY AND CONCLUSION

Acute Leukemias are hemopoietic neoplasms characterized by accumulation of blast cells in the bone marrow and peripheral blood.

AML also known as acute myelogenous leukemia or acute nonlymphocytic leukemia (ANLL), is a cancer of the myeloid line of blood cells, characterized by the rapid growth of abnormal white blood cells that accumulate in the bone marrow and interfere with the production of normal blood cells. AML is the most common acute leukemia affecting adults, and its incidence increases with age. Although AML is a relatively rare disease, accounting for approximately 1.2% of cancer deaths in the United States, its incidence is expected to increase as the population ages. AML is confirmed by an excess of primitive blast cells in the bone marrow required to be at least 20% and there is 8 subtypes of AML. Treatment and prognosis varies among subtypes. Five year survival varies from 15–70%, and relapse rate varies from 33–78%, depending on subtype. AML is treated initially with chemotherapy aimed at inducing a remission; patients may go on to receive additional chemotherapy or a hematopoietic stem cell transplant. Recent research into the genetics of AML has resulted in the availability of tests that can predict which drug or drugs may work best for a particular patient, as well as how long that patient is likely to survive.

ALL or acute lymphoid leukemia is an acute form of leukemia, or cancer of the white blood cells, characterized by the overproduction of cancerous, immature white blood cells—known as lymphoblasts. In persons with ALL, lymphoblasts are overproduced in the bone marrow and continuously multiply, causing damage and death by inhibiting the production of normal cells—such as red and white blood cells and platelets—in the bone marrow and by spreading (infiltrating) to other organs. ALL is most common in childhood with a peak incidence at 2–5 years of age, and another peak in old age. ALL may be B or T lineages and are classified by FAB classification into L1, L2, L3. The earlier ALL is detected, the more effective the treatment. The aim is to induce a lasting remission, defined as the absence of detectable cancer cells in the body (usually less than 5% blast cells in the bone marrow).

Morphology remains the method by which acute Leukemia is initially detected and is the major aid with cytochemical reaction and immunophenotyping in distinguishing between ALL and AML.

The rennin-angiotensin system (RAS) is a bioenzymatic cascade that plays an integral role in cardiovascular homoeostasis by influencing vascular tone, fluid and electrolyte balance and the sympathetic nervous system.

The biological actions of the RAS are mediated primarily by the highly active octapeptide angiotensin II (Ang II). Traditionally, the RAS was viewed as a circulating endocrine system, whereby renin released from the juxtaglomerular cells of the kidney cleaves the liver-derived macroglobulin precursor angiotensinogen, to produce the inactive decapeptide angiotensin I, which is then converted to the active octapeptide Ang II by angiotensin converting enzyme (ACE) within the pulmonary.

There is increasing evidence that Ang II, a major regulator of blood pressure and cardiovascular homeostasis, is involved in the regulation of cell proliferation, angiogenesis, inflammation and tissue remodeling, which suggests that this peptide might also play a role in cancer.

Components of RAS are expressed in several adult organs including the liver, kidney, pancreas, brain and reproductive organs. It is the paracrine mechanisms of locally expressed RAS, not in its circulating counterpart, that appear important for tumorigenesis. Ang II is the main effector of the RAS and it alternatively binds to either Ang II T1R or Ang II T2R. The Ang II T1R and Ang II T2R can act as antagonists, and mediate effects on cell migration and proliferation of metastatic cancer cells and hemopoietic stem-progenitor cells. Components of the RAS are frequently differentially expressed in various cancers in comparison with their corresponding non- malignant tissue.

The Ang IIT1R belongs to the seven transmembrane class of G-protein-coupled receptors. Four cysteine residues are located in the extracellular domain, which represent sites of disulphide bridge formation and are critical tertiary structure determinants. The transmembrane domain and the extracellular loop play an important role in Ang II binding. The binding site for Ang II is different from the binding site for Ang IIT1R antagonists, which interacts only with the transmembrane domain of the receptor. Like most G-protein-coupled receptors, the Ang IIT1R is also subject to internalization when stimulated by Ang II.

Studies of knockout mice for ACE as well as other RAS components such as angiotensinogen, rennin, Ang II T1R and Ang II T2R have further implicated a regulatory role for the RAS in hematopoiesis. These mice have exhibited not only phenotypes related to blood pressure, but also demonstrated defects in development and in hematopoietic system. ACE null mice are mildly anemic, so it is presumed that the lack of systemic or local production of Ang II has a detrimental effect on erythropoiesis. Yet, the RAS has not been fully elucidated in patients with acute Leukemia.

For this reason, this study aimed to investigate serum level of Ang IIT1R and the soluble ACE (CD143) in patients with Acute Leukemia in order to extrapolate their possible prognostic value.

Individuals submitted to this study were divided into three groups:

Group I: Involved 20 healthy volunteers clinically free from hypertension or sarcoidosis (control group), their mean age was 33.25years 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 27 patients with newly diagnosed AML.

Group III: Involved 10 patients with newly diagnosed ALL.

Patients in group II and III were of matched age as the control group and were recruited from Hematology department, MRI, Alexandria University and clinical research center, Faculty of Medicine, Alexandria University. An informed consent was taken from all contributors in this study.

Patients with AML received 3 + 7 protocol of induction including: Daunomycin 45 mg/m2 for 3 days, Cytosine arabinoside 100 mg/ m2 x2 / day for 7 days. Patients with ALL received induction protocol as follows: Vincristine 1.4 mg/ m2 days 1, 8, 15, 22, Prednisolone 1 mg / kg / day x 28 days, Doxorubicin 25 mg/ m2 days 1, 2, 3. After completion of the cycles and restoration of bone marrow cellularity, bone marrow aspiration was done. Patients who achieved complete remission had a BM blasts less than 5%. Those who did not achieve complete remission received a 2nd induction cycle.

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 and mean values ± SE.

The results showed that the activity of ACE (U/L) and the concentration of Ang IIT1R (U/L) in patients groups with either AML or ALL before therapy were significantly higher than in control group. After therapy, the activity of the enzyme and its receptor concentration in both groups of patients were significantly decreased but still significantly higher than in normal control subjects.

Moreover, the mean values of indirect bilirubin (mg/dl), total bilirubin (mg/dl) and SGPT (u/l) concentrations in both groups of patients were significantly higher than normal control group. In addition, the concentrations of direct bilirubin (mg/dl) as well as SGOT (u/l) in patients with ALL were significantly higher than normal control group. On the other hand, the mean values of albumin(mg/dl), creatinine (mg/dl), sodium (meq/l), potassium (meq/l), calcium (meq/l) and phosphorus (meq/l) concentrations in both groups of patients were significantly lower than in normal control group.

On the other hand, the mean value of WBCs count (x10e9) in both groups of patients were higher than in control group. On the other hand, Platelets count and Hb concentration in both groups of patients were significantly less than in normal subjects. Also, the blast cells % in patients with either AML or ALL before therapy was significantly higher than in control group. After therapy, the % of blast cells in both groups of patients were significantly decreased than their corresponding values before therapy and still significantly higher than in control group.

In AML patients group, ACE activity (U/L) showed a significant positive correlation with blast cells % at presentation. While in ALL patients groups, it was noticed that there was a significant positive correlation with blast cells % and Hb concentration (g/dl), and was inversely correlated with Phosphorus concentration (meq/l).

In addition, Ang IIT1R concentration (U/L) of AML patients group showed a significant positive correlation with blast cells % and urea concentration (mg/dl) at presentation. While in ALL patients groups, it was noticed that it was a significantly positive correlated with blast cells %, and was inversely correlated with WBCs count (x10e9) and Sodium concentration (meq/l).

The ROC curves analysis was used to compare the diagnostic values of serum ACE activity (U/L) and Ang IIT1R concentration (U/L) depending on the area under the curves (AUC). The higher AUC corresponds to a better diagnostic test. The curve showed that both parameters were excellent diagnostic markers of acute leukemia as was indicated by asymptomatic significance and very high area under the curve (100%, 100% respectively).

The optimum cut off value of ACE activity (U/L) and Ang IIT1R concentration (U/L) were with a corresponding sensitivity 100% and specificity 100% for both parameters.

To study the prognostic values of these two parameters the Kaplan-Meier disease free survival (DFS) curves were constructed. For the first time, we could define a prognostic value for estimating the levels of ACE activity (U/L) and Ang IIT1R concentration (U/L) in patients with acute leukemia. The Kaplan-Meier disease free survival curve specified a cut off level for ACE < or > than 50.25 U/L with statistical significance ($p < 0.00$). This was true for Ang IIT1R as a cut off value of below or above 386 U/L could discriminate between a higher mean disease free survival of 24 months compared to 14 months in patients with higher level above the cut off point and the difference was statistically significant ($p < 0.001$) and this patients necessitating a more aggressive induction chemotherapy protocol

Conclusion

To the best of our knowledge the above study is the first to investigate the levels of serum ACE and its soluble receptor1 in patients with acute leukemia. We may conclude that estimating the serum level of ACE and soluble Ang IIT1R might be of informative diagnostic and prognostic value.

The findings of the present study make AR blockers and ACE inhibitors as a targeted option in the management of acute leukemia.

Recommendations

From the results of the present study we recommend the following:

1– Estimation of Ang II T1R and ACE levels in acute leukemia is of value in deciding the treatment protocol.

2– Further study on the other components of the RAS system is warranted.

3– Further studies on the impact of RAS on other hemopoietic neoplasms are to be envisaged namely the chronic leukemias and lymphomas.

4– Novel clinical applications of ACE inhibitors and Ang II T1R blockade in the management of acute leukemia is worthwhile.