Research Article

Beclin-١ Gene Expression in Patients with Chronic Myeloid Leukemia

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Abstract
Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm, the introduction of imatinab as one of the tyrosine kinase inhibitors (TKI) in the treatment of CML represent the most successful example of targeted therapy in human cancers. Autophagy is the process of cellular degradation in which cytoplasmic organelles are sequestered by autophagosome and then degraded inside lysosomes. The aim of this study was to assess beclin-١expression as a marker of autophagy in CML, and to evaluate the effect of imatinab therapy on its expression**.** Our study included ٥٠ subjects, ٣٥ patients newly diagnosed with CML and ١٥ controls. They were assessed for beclin-١gene expression before and four weeks after treatment with imatinab. Our results showed a statistically significant increase in beclin-١ gene expression results before treatment in patient groups. We concluded that beclin-١ gene expression as a marker of autophagy is increased after imatinab therapy in CML patients, indicating induction of autophagy.
Keywords: Chronic myeloid leukemia, Imatinib, Autophagy, ATG

Introduction
Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm, characterized by expansion of pluripotent bone marrow stem cells. The hallmark of the disease is the presence of a reciprocal t (٩;٢٢) (q٣٤; q١١.٢), resulting in a BCR-ABL fusion gene and production of a BCR-ABL fusion protein; BCR-ABL has constitutive tyrosine kinase activity and is necessary and sufficient for production the disease[١].

The introduction of imatinab as on of the tyrosine kinase inhibitors (TKI) in the treatment of chronic myeloid leukemia represent the most successful example of targeted therapy in human cancers[٢]. Imatinib is a chemotherapeutic agent that specifically binds to the adenosine triphosphate binding pocket of the BCR/ABL fusion protein. This binding inhibits the subsequent phosphorylation events of the target proteins and suppresses cell proliferation[٣].

Autophagy is derived from Greek, where "auto" means one self and "phagy", means to eat. It refers to the cellular degradation process in which cytoplasmic organelles are sequestered by autophagosome and then degraded inside lysosomes[٤].

Autophagy can be induced by a number of stressors, acting to degrade protein polymers, oxidized lipids, injured organ-elles, as well as intracellular pathogens [٥]. It is related with a series of neurodegenerative diseases, liver diseases, myopathy, tumor progression, aging, infection, immunization and inflammatory diseases[٤].

The formation of autophagosome, including nucleation and expansion of the autophagosomal membrane, is dependent on the activity of ATG (autophagy-related) proteins, including the Beclin-١ complex, the ATG) ١٢ and light chain ٣ systems[٦].

Autophagic degradation had long been regarded as bulk and non-selective, now it is known that autophagy is more useful as it can also selectively degrade various targets, including protein aggregates, damaged mitochondria, and even intracellular pathogens and this is called selective autophagy. In most cases, selectivity is determined by receptor protein[٧].

Beclin-١ is expressed in many human and murine tissues, and is localized primarily within cytoplasmic structures, including the endoplasmic reticulum, mitochondria and the perinuclear membrane[٨]. Beclin-١ and its binding partner class III phosphoinositide ٣-kinase (PI٣K), also named Vps (vacular protein sorting) ٣٤, are required for the initiation of the formation of the autophagosome[٨].

Mutations of the Beclin-١ interfer with its abilities to promote nutrient deprivation-induced autophagy and suppress tumorigenesis[٦].

Reduced beclin-١ gene expression was found in many cancers including esophageal, ovarian and lung cancer cells. On the other hand, there is increased expression in other cancers including colorectal and gastric cancers[٩].

BCR-ABL signaling, leads to activation of the mammalian of rapamycin (mTOR). Inhibition of BCR-ABL by TKIs has now been shown to not only induce apoptosis but also autophagy a similar effect to that seen after growth factor with drawal[٢].

The presence of leukemic stem cells which are insensitive to TKIs and contribute to the persistence of disease by representing a reservoir of self-renewing cells that replenish the disease after drug discontinuation. This finding has refocused the interest of scientist towards drug combinations treating with TKIs and simultaneously targeting alternative survival mechanisms[٢].

Subjects and Methods

The study population included ٥٠ subjects , ٣٥ newly diagnosed chronic myeloid leukemia patients were selected from South Egypt Cancer Institute and fifteen apparently healthy subjects in the period from June ٢٠١٤ to April ٢٠١٦ and were followed up for four weeks after imatinab therapy. Group I included ١٧ patients newly diagnosed with chronic myeloid leukemia and they were followed up four weeks after treatment with imatinab. Their ages ranged from ٣٠ to ٦٧ years and this group included ١٣ males and ٤ females. Group II included ١٨ CML patients in accelerated phase, they were followed up four weeks after treatment with imatinab. Their ages ranged from ٣١ to ٧٠ years and this group included ١١ males and ٧ females. Group III (control group) included ١٥ apparently healthy subjects matched for age and sex for patient group and their ages ranged from ٣٠ to ٧٠ years and this group included ١٠ males and ٥ females. Both patients and control groups were subjected to the following: complete history taking, clinical examination and laboratory investigations:

A) Routine investigations: including CBC determined by automated cell counter (Cell Dyn ٣٥٠٠, Abbott Diagnostics, USA), ESR determined by Westergren method, RBG and renal function tests including (urea, creatinine and uric acid) analyzed by (Integra ٤٠٠ plus, Roche Diagnostics, German).

B) Diagnostic investigations: ١-Examination of Leishman-stained peripheral blood smears for differential leucocytic count and assessment of blast cell number. ٢- Bone marrow aspiration by marrow punctures needles (Klima type) either from anterior or posterior superior iliac spine and examination of leishman-stained smears. ٣-BCR-ABL gene expression detection by quantitative RT-PCR (Light cycler® Roche, German).

C) Special investigations: Beclin-١ gene expression detection by quantitative RT-PCR using (Light cycler® Roche, German).

Results

The present study included ٣٥ CML patients and ١٥ apparently healthy volunteers as a control group.

All results are summarized in tables (I-III) and figures (١-٣). Demographic data of the studied groups are shown in (table I). Comparison between TLC, HB level and platelet count before and after treatment in group I as shown in (table II).

There was high statistically significant decrease in TLC when comparing results before and after treatment, (P value = <٠.٠٠١), also there was statistically significant decrease in platelet count, (P value = ٠.٠٠٣), while, there was no statistically significant difference between

HB level before and after treatment, (P value = ٠.٠٦٨).

Comparison between TLC, HB level and platelet count before and after treatment in group I as shown in table (III).
There was high statistically significant decrease in TLC when comparing results before and after treatment, (P value = <٠.٠٠١), also, there was statistically significant increase in HB level after treatment, (P value= ٠.٠٢٧), while, there was no statistically significant difference between platelet count before and after treatment, (P value = ٠.١٠٢).

Comparison between beclin-١ gene expressions in the studied groups as shown in (figure ١).

There was high statistically significant increase in beclin-١ gene expression results before treatment in group when compared with group III (P value = ٠.٠٠١) & moderate statistically significant increase in its results before treatment in group II when compared with group III (P value=٠.٠٠٦).

Regarding beclin-١ gene expression results after treatment, there was no statistically significant difference between group I & group II (P value = ٠.٦٣١).

Regarding beclin-١ gene expression results within each group before and after treatment, there was high statistically significant increase in beclin-١ gene expression results after treatment, (P value = < ٠.٠٠١) in both group I & II.

Correlations between beclin-١ gene expression before treatment and BCR-ABL gene expression results in group I & II as shown in (figures ٢ & ٣).

There was high statistically significant negative correlation between beclin-١ gene expression before treatment and BCR-ABL gene results (r = -٠.٨٤٤ & -٠.٨٢٢) in group I and II respectively.

Table (I): Demographic data of the studied groups

	Group I (Chronic) (n=١٧)	**Group II (Accelerated) (n=١٨)**	**Group III (Control) (n=١٥)**	**P value**		
Age (years)				٠.٩٧٥		
Range	(٣٠-٦٧)	(٣١-٧٠)	(٣١-٧٠)	**I vs II**	**I vs III**	**II vs III**
Mean ± SD	٥٠.٥±١١.٩	٥٠.٣±١٠.٢	٤٩.٧±١١.٢	٠.٩٩٨	٠.٩٧٤	٠.٩٨٦
Sex				٠.٦١٧		
Male	١٣ (٧٦.٥٪)	١١ (٦١.١٪)	١٠ (٦٦.٧٪)	**I vs II**	**I vs III**	**II vs III**
Female	٤ (٢٣.٥٪)	٧ (٣٨.٩٪)	٥ (٣٣.٣٪)	٠.٣٢٨	٠.٥٣٨	٠.٧٤١

: significant difference at p value < ٠.٠٥

Table (II): Comparison between TLC, HB level and platelet count before and after treatment in group I

Group I (Chronic phase) (n=١٧)	**Before treatment**	**After treatment**	**P value**
TLC (× ١٠³ cell/ µl)			<٠.٠٠١*
Range	٢٥-٦١٤)	(٤.٧-١١)	
Mean ± SD	١٦٢.٩±١٥٧.٣	٨.١±٢.١	
HB (g/dl)			٠.٠٦٨
Range	(٧.٤-١٣)	(١٠.٥-١٤.٢)	
Mean ± SD	١٠.٥±١.٧	١١.٩±١.٢	
Platelet count (× ١٠³/ µl)			٠.٠٠٣*
Range	(١٣٤-١٠٠٠)	(١٤٥-٤٠٩)	
Mean ± SD	٥٠٦.٤±٢٤١.٤	٢٨٤.٤±٩١.٠	

Table (III): Comparison between TLC, HB level and platelet count before and after treatment in group II

Group II (Accelerated Phase) (n=١٨)	Before treatment	After treatment	P value
TLC (× ١٠³ cell/ µl) Range Mean ± SD	(٣٦-٨٤٣) ٢٧١.١±٢٤٩.٩	(٤.٦-١٠) ٧.٥±١.٨	<٠.٠٠١*
HB (g/dl) Range Mean ± SD	(٧.٢-١٣.٥) ١٠.٨±١.٥	(١٠.٨-١٥) ١٢.٥±١.٠	٠.٠٢٧
Platelet count (× ١٠³/ µl) Range Mean ± SD	(٤٣-٦٧٠) ٣٢٧.٩±١٨٦.٧	(١٧٠-٤٢٠) ٢٤٩.١±٨٣.٤	٠.١٠٢

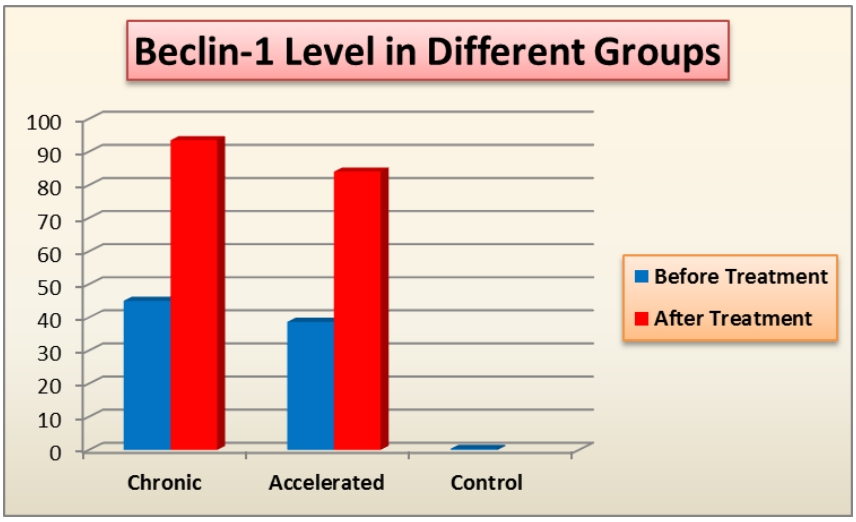

Figure (١): Comparison between beclin-١ gene expression results in the studied groups.

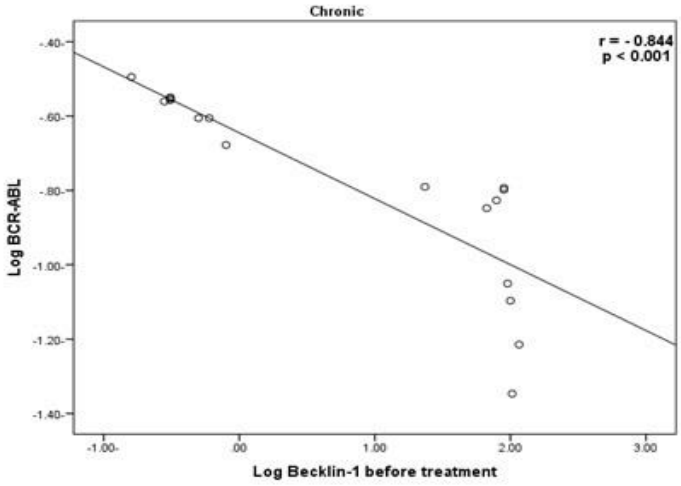

Figure (٢): Correlation between beclin-١ gene expression before treatment and BCR-ABL gene expression in group I

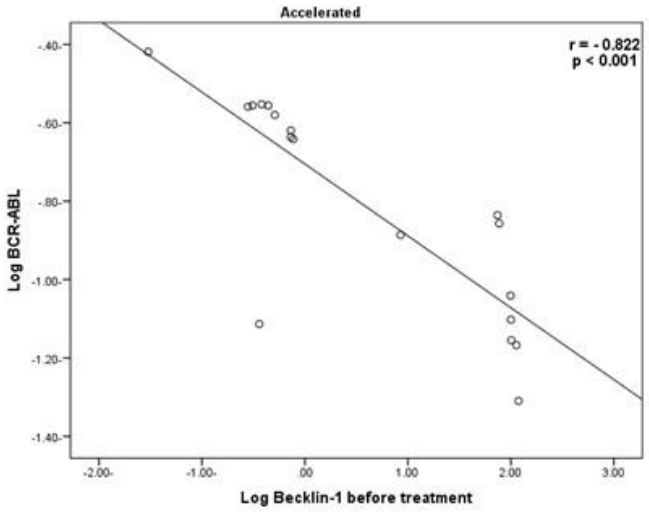

Figure (٣): Correlation between beclin-١ gene expression before treatment and BCR-ABL gene expression in group II

Discussion

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm, characterized by expansion of pluripotent bone marrow stem cells. The hallmark of the disease is the presence of a reciprocal t (٩;٢٢) (q٣٤; q١١.٢), resulting in a BCR-ABL fusion gene and production of a BCR-ABL fusion protein; BCR-ABL has constitutive tyrosine kinase activity and is necessary and sufficient for production of the disease[١].

Autophagy is a recycling process that leads to sequestration and degradation of damaged proteins and other intracellular material within lysosomes[١٠]. During autophagy, portions of the cytoplasm, protein aggregates or organelles, are sequestered within double-or multi-membraned vesicles called autophago-somes, and subsequently delivered to lysosomes for degradation[١١].

In the present study ٣٥ newly diagnosed CML patients were assessed for autophagy by beclin-١ gene expression detection in their blood, then reassessment was done after beginning of therapy with imatinab.

All patients in this study (group I & II) developed complete hematologic response, which was assessed by peripheral blood counts, peripheral blood smears and clinical evaluation, while Medhi et al., (٢٠١٠)[١٢] reported that ٩٥٪ of patients developed complete hematologic response.

In the current study, there was highly significant negative correlation between beclin-١ gene expression before treatment in group I and BCR-ABL gene expression results, that was in accordance with Vignir et al, (٢٠١١)[٢], who reported that BCR-ABL signaling leads to activation of the PI٣K/AKT pathway and mTOR with subsequent decreasing level of autophagy.

In the present study, there was highly significant increase in beclin-١ gene expression detection after beginning of the treatment with imatinab which indicate induction of autophagy. That is in agreement with Geylani et al., (٢٠١١)[١٣], who demonstrated that there were dose dependent increases in expression levels of ATG٥ and beclin-١ genes in K٥٦٢ cells exposed to ٠.١, ١, and ١٠ nM imatinib, respectively. Also, this is consistent with Vignir et al., (٢٠١١)[٢], who reported that inhibition of BCR-ABL by TKIs has now been shown not only to induce apoptosis but also autophagy, a similar effect to that seen after growth factor withdrawal.

In agreement with the present study, Calabretta & Salomoni, (٢٠١٢)[١٤] demon-strated that imatinab as one of TKIs induce autophagy and that combining imatinab

with CQ, as an autophagy inhibitor, can potentiate the effect of TKI & restoring the sensitivity to imatinab.

Conclusion

Beclin-١ gene expression as a marker of autophagy is increased after imatinab therapy in CML patients, indicating induction of autophagy.

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