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RESEARCH ARTICLE

IMPACT OF T315I MUTATION ON THE OUTCOME OF IMATINIB-RESISTANT CML IN EGYPTIAN PATIENTS

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Abstract

Background: BCR-ABL T315I BCRT315I mutation is one of the most frequently identified mutations in Imatinib-resistant chronic myelogenous leukemia (CML) patients. However, its clinical impact remains controversial.

Objectives: to assess the clinical characteristics and outcome of T315I mutation in 79 Egyptian patients with CML after failure of Imatinib therapy.

Results: T315I mutation was present in 14 (18%) patients. The level of resistance was heterogeneous. Forty nine patients were in chronic phase, 7 in accelerated phase, and 23 in blast crisis. Imatinib dose escalation in 2 resistant patients with T315+ showed transient good response. Responses were also observed in 3 out of 8 patients who were shifted to 2nd generation Tyrosine Kinase Inhibitor (TKI) after Imatinib resistance. There was no difference in patient characteristics and survival between T315I⁺ and T315I⁻ patients.

Conclusion: the survival of T315I⁺ patients remains mainly dependent on the disease phase and the response duration. The difference in the disease outcome from most of previously reported studies may be related to mutation heterogeneity and to different ethnicity of patient population.

Evaluation of chromosomal abnormalities other than Ph¹, and additional mutation at T315I identification is needed to know if there are other mechanisms of resistance.

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Introduction:-

The Philadelphia (Ph¹) chromosome is the hallmark of chronic myeloid leukemia (CML), it represents the fusion of the Abelson murine leukemia (ABL) gene in chromosome 9 with the breakpoint cluster region (BCR) gene in chromosome 22. This fused *BCR-ABL* oncogene encodes a deregulated, constitutively active tyrosine kinase that promotes growth, proliferation and apoptosis resistance.

Typically, CML has two or three phases: the disease is usually diagnosed in chronic phase (CP), followed by – with or without an acceleration phase (AP) – a blastic phase or crisis (BC). In the absence of specific treatment, the natural evolution of the disease is the progression to a fatal BC.⁽¹⁾

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Imatinib Mesylate (IM), the front-line treatment of CML is a selective tyrosine kinase inhibitor (TKI) that targets theadenosine triphosphate (ATP) binding site of the BCR-ABL protein, which results in the inhibition of phosphorylation of proteins involved in BCR-ABL signal transduction leading to growth arrest and apoptosis of the hematopoietic cells that express BCR-ABL without affecting the normal cells.⁽²⁾ Unfortunately, failure to respond to IM develops in some patients (primary resistance). In addition, approximately 15% of IM-treated patients, under the selective pressure of TKI, acquire recurrent BCR-ABL amino-acid exchanges⁽³⁾ which modify the conformation of IM binding site, preventing IM binding and result in decrease sensitivity of the drug, at different levels, and disease progression (acquired resistance).^(4,5)

T315I BCR-ABL mutation remains one of the most frequently detected mutations in CML TKI-resistant patients.⁽⁶⁾ In patients bearing this mutation, IM resistance appears to be heterogeneous. It confers high levels of IM resistance *in vitro*, but different studies report a controversial clinical impact. Several studies suggested that the presence of a T315I mutation is associated poor survival.^(7,8) Conversely, other studies suggested no statistically significant survival difference between resistant patients with T315I mutation and those with other or no mutations.⁽⁹⁾ In addition, it is of crucial clinical importance to know whether IM dose escalation may – at least partially – overcome resistance and improve the disease outcome.

Although the importance of T315I mutation is increasing nowadays, published literature focusing on survival information for Egyptian patients with CML harboring this mutation remains very limited.

The aim of this study was to assess the clinical characteristics of Egyptian patients with CML harboring T315I mutation, and to evaluate their outcome after failure of IM therapy.

Subjects:-

The study included 79 CML Egyptian patients *resistant* to imatinib (IM) therapy between November 2010 and December 2016, regardless of the time of diagnosis and start of IM therapy.

All patients were in CP at diagnosis and had received the standard daily IM dose of 400 mg. Patients who had received allogeneic hematopoietic stem cell transplantation were excluded from our study.

IM resistance was defined according European LeukemiaNet (ELN) guidelines⁽¹⁰⁾ as failure of achievement of either Complete Hematologic Response (CHR) after 3 months, Complete Cytogenetic Response (CCyR) at 12 months and Major Molecular Response (MMR) at 18 months of IM initiation (primary resistance), or loss of previously achieved hematologic, cytogenetic or molecular response (acquired resistance).

Among these 79 patients, 9 patients had failure to achieve CHR at 3 months and 23 patients had failure to achieve CCyR and/or MMR at 18 months; 10 patients had loss of CHR and CCyR and 7 patients had loss of CCyR; and 30 patients had progression to AP/BC.

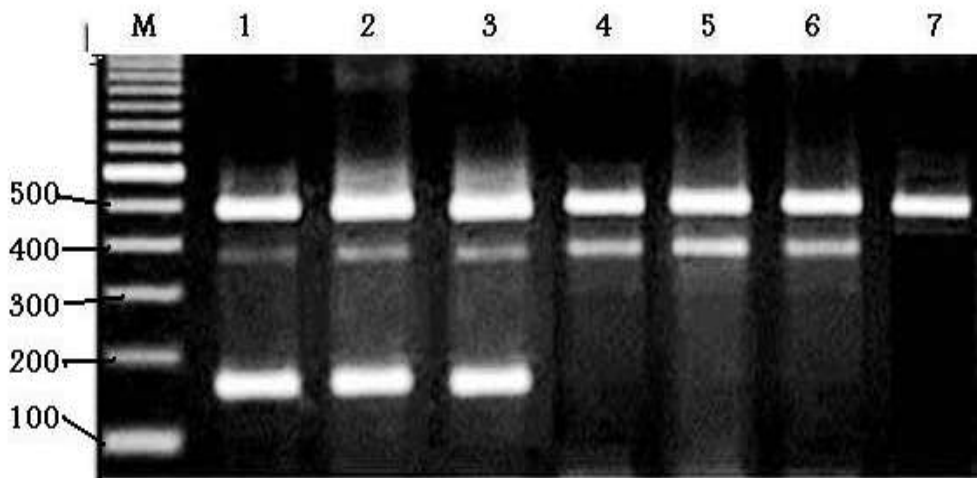
An informed written consent was obtained from all participants prior to their inclusion in the study protocol, according to the ethical guidelines of the Medical Research Institute Alexandria University (Appendix1, Informed Written Consent for patient participation in a Clinical Research 2011)

Statistical Analysis:-

The chi-square test was used to determine the significance between variables. $P < 0.05$ was considered statistically significant. Survival was analyzed according to Kaplan-Meier methods⁽¹¹⁾ and Log rank test was used to compare between groups. Overall Survival (OS) was calculated from the time of Imatinib failure until date of death or last follow up. All statistical analyses were conducted with SPSS Version 24.0. Released 2016 (SPSS IBM Corp., Armonk, NY, USA), <https://www01.ibm.com/support/docview.wss?uid=swg21476197>.

Methods:-

T315I BCR-ABL gene Mutation was performed to all studied CML patients at the time of IM resistance, using allele specific oligonucleotide - polymerase chain reaction (ASO-PCR).⁽¹²⁾ Total RNA was extracted from whole blood using ABIOPure™ total RNA extraction kit (Alliance Bio, USA). Complementary DNA (cDNA) was generated by SuperScript III cDNA synthesis kit (Invitrogen, CA, USA) following the manufacturer's instructions. Sequence of forward and reverse primers for wild type ABL (WT), T315I mutant type (MT) and Internal control (β -actin) used for ASO-PCR was adapted from previous report.[12] Sequences were specifically amplified in a PCR reaction. The amplified products were detected and assessed by electrophoresis on 2% ethidium bromide-stained agarose gel. The PCR products of T315I mutant, T315 wild type (WT) and β -actin gene were 158 bp, 374 bp and 540 bp respectively (Figure 1).



M: molecular size maker 100bp. Lanes 1-3 patients positive for T315I mutation. Lanes 4-6 patients negative for T315I mutation. Lane 7: internal control actin gene.

Figure 1:- Agarose gel electrophoresis of PCR products for detection of T315I mutation

Results:-

Fourteen patients (18%) were harboring T315I gene mutation (T315I⁺) and 65 patients (82%) hadn't the mutation (T315I⁻), but they might harbor other mutation(s). The patients' characteristics at time of IM failure and the clinical outcome are summarized in Table [1]. There was no significant difference in characteristics between T315I⁺ and T315I⁻ patients' groups.

The best Response before treatment Failure:-

Before treatment failure, 10 (71%) patients with T315I mutation achieved CHR, CCyR and MMR while 2 patients (14%) achieved CHR only. For T315I⁻ patients, 37 (57%) patients achieved CHR, CCyR and MMR; while 21 (32%) patients achieved CHR only. There was no significant relationship between the mutation status (presence or absence of T315I) and CCyR to treatment ($p=0.244$) [table 2].

Mutation status, response duration and the phase of the disease at Imatinib failure:-

Our study demonstrated no significant relationship between the mutation status and the phase of the disease at IM failure ($p=0.828$) [table 3]. The Median duration of response (from start of treatment until treatment failure) in T315I⁺ and T315I⁻ patients were 32 and 33.5 months respectively and no significant relationship was also found between the mutation status and duration of treatment response ($p=0.433$) [table 4].

Table 1:- Characteristics of CML resistant patients at time of Imatinib failure and disease outcome .

Characteristic	T315I+ (n = 14)	T315I- (n = 65)	p value
Patients, No. (%)	14 (18%)	65 (82%)	
Mean Age, years (range)	48 (33-65)	46 (25-69)	
Male/Female	6/8	36/29	0.143
Clinical features			
- Splenomegaly, no. (%)	12 (86)	63 (97)	0.847
- Fatigue, no. (%)	11 (79)	62 (95)	0.624
- Fever, no. (%)	06 (43)	35 (54)	0.847
- Bone pain, no. (%)	06 (43)	40 (62)	0.536
- Night sweats, no. (%)	07 (50)	36 (55)	0.392
- Bleeding, no. (%)	05 (36)	27 (42)	0.759
- Hepatomegaly, no. (%)	03 (21)	23 (35)	0.662
Hematological parameters			
- Mean Hb level, g/dl (range)	11.4 (8.5-14.2)	11.1 (7.2-17.0)	0.781
- Mean TLC x10 ⁹ /L (range)	52.9 (5.9-139.5)	28.8 (4.0-105.0)	0.358
- Mean Basophil% (range)	4.3 (0.0-15.0)	2.8(0-12.0%)	0.366
- Mean Blast% (range)	11.7(1.0-43.0)	11.8 (0.0-90.0)	0.072
- Mean Platelet count, x10 ⁹ /L (range)	323.6 (23.0-890.0)	308.5 (80.0-1254.0)	0.777
Type of IM Resistance			
- Primary, No (%)	04 (29)	28 (43)	0.162
- Secondary, No (%)	10 (71)	37 (57)	
CML phase at Imatinib failure			
- Chronic, no. (%)	08 (57)	41 (63)	0.828
- Accelerated, no. (%)	01 (07)	06 (09)	
- Blastic phase, no. (%)	05 (36)	18 (28)	
Median response duration, months (range)	32 (12 – 62)	33.5 (3 - 70)	
Disease Outcome			
- No. dead (%)	10 (43)	29 (35)	0.063
- No. alive (%)	04 (57)	36 (65)	

Abbreviations: Hb = Hemoglobin; TLC= Total leucocyte count

Table 2:- Relation between Mutation status and Cytogenetic Response

Response achieved	T315I Mutation status				Total (n=79)		P
	T315I ⁺ (n=14)		T315I ⁻ (n=65)				
	No	%	No	%	No	%	
CCyR at 12 months	10	71	37	57	49	62	0.244*

Abreviation: CCyR= Complete Cytogenetic Response

*Non-significant

Table 3:- Relation between Mutation status and disease phase at time of imatinib failure

CML Phase	T315I Mutation status				Total (n=79)		P
	T315I ⁺ (n=14)		T315I ⁻ (n=65)				
	No	%	No	%	No	%	
Chronic Phase	08	57	41	63	49	62	0.828*
Advanced stage	06	43	24	37	30	38	

*Non-significant

Table 4:- Relation between Mutation and Duration of treatment response

Duration of response	T315I Mutation status				Total (n=79)		<i>P</i>
	T315I ⁺ (n=14)		T315I ⁻ (n=65)				
	No	%	No	%	No	%	
< 1 year	02	14	06	09	08	10	0.828*
≥1year	12	86	59	91	71	90	

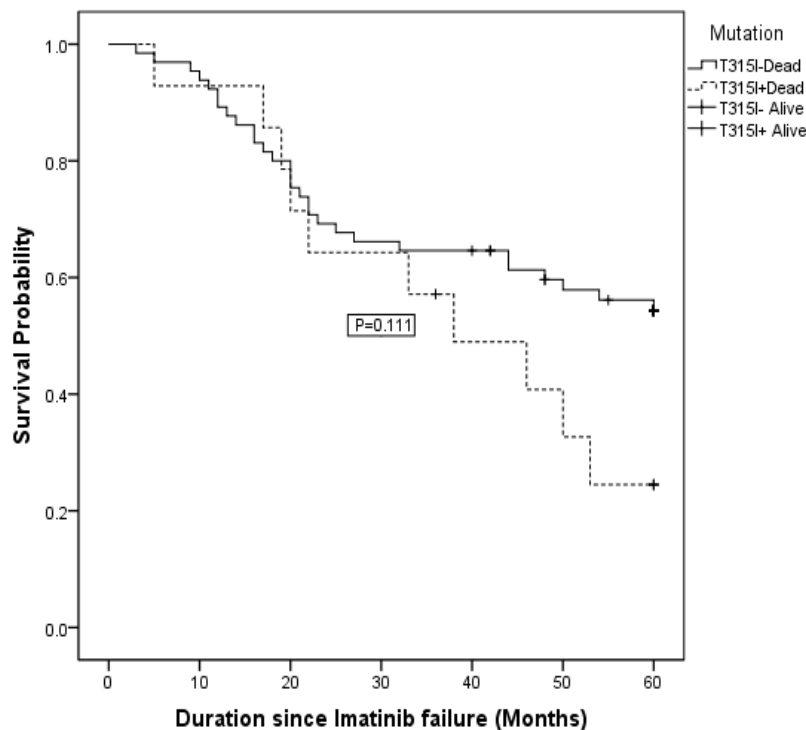
*Non-significant

Overall survival (OS):-

After a median follow-up of 42 months from IM failure, the estimate mean OS of all the studied CML patients with IM resistance was 42.8 months. Patients' OS from the time of IM failure according to the mutation status (presence or absence of T315I), phase of the disease at the time of IM failure, and duration of treatment response were shown in Figures 2A through C.

The estimate mean OS of T315I⁺ and T315I⁻ patients were 38.2 and 43.7 months with 95% CI of 28.6-47.9 and 38.6-48.8 months respectively (figure 2-A) with no significant difference in survival between the 2 groups of patients ($p=0.111$). Thirty nine of 79 patients (49.4%) died, including 11 of 49 (22.4%) patients in CP, 5 of 7 (71.4%) patients in AP, and all 23 patients (100%) in BC. In CP, AP and BC, the estimate mean OS from IM failure were 56.9, 32.7 and 15.7 months respectively with highly significant difference ($p<0.001$). Advanced disease phase (AP and BC) is significantly associated with poor survival (figure 2-B).

The estimate mean OS for patients with treatment response duration less than one year and ≥ 1 years were 31.1 and 44.1 months with 95% CI of 22.0-40.3 and 39.3-49.0 months respectively with significant difference ($p=0.04$), short IM response duration is associated with poor survival (figure 2-C).

**Figure 2-A:-** Survival of resistant patients since IM failure according to the mutation status.

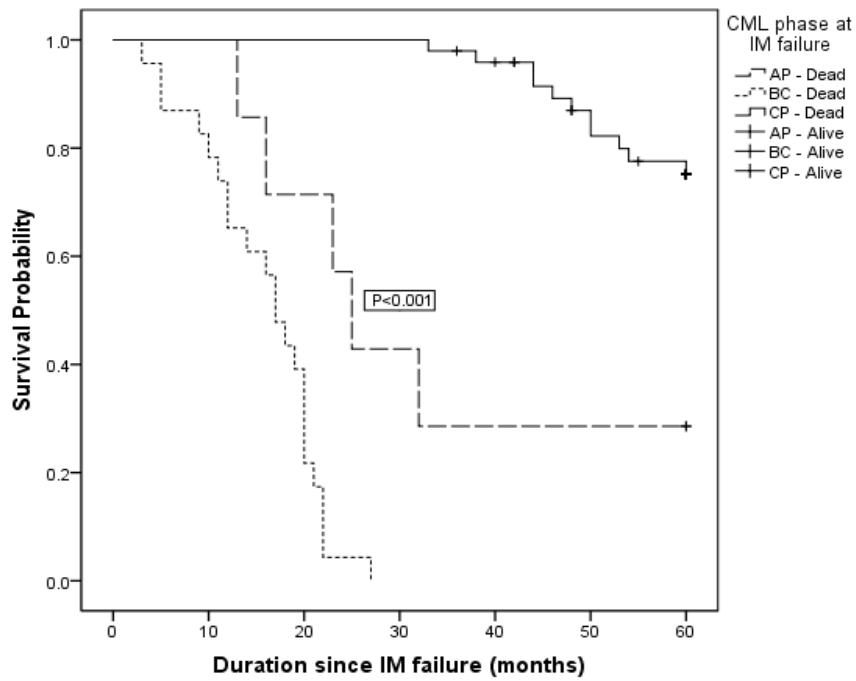


Figure 2-B:- Survival of resistant patients since IM failure according to the phase of the disease at IM failure

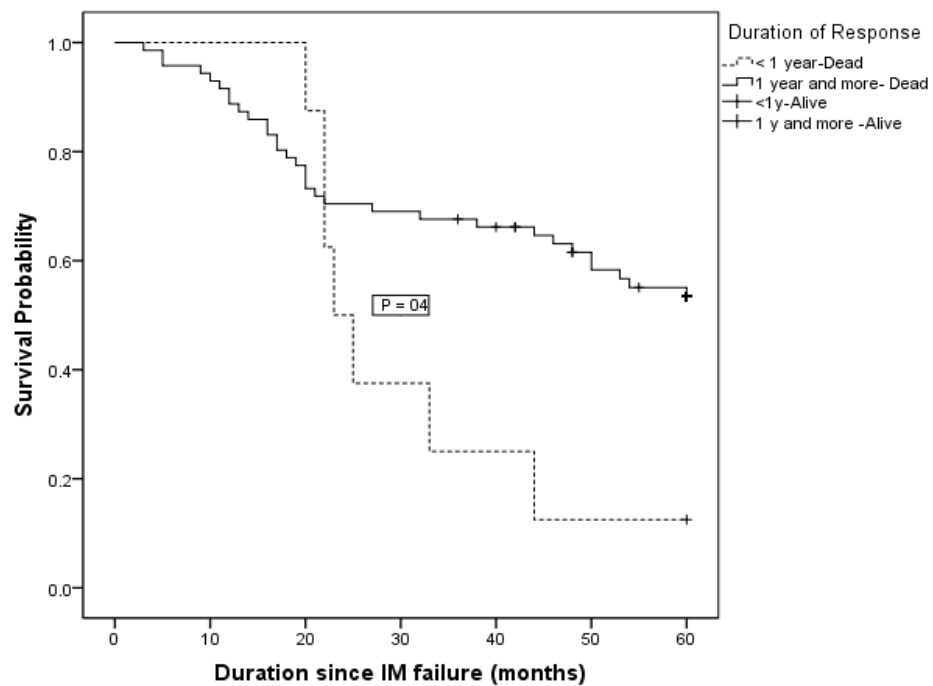


Figure 2-C:- Survival of resistant patients since IM failure according to the duration of treatment response.

Discussion:-**Frequency of T315I Mutation:-**

We present our experience for T315I mutation among 79 CML Egyptian patients who failed IM therapy and we identified the mutation in 14 patients (18%).

The reported T315I mutation frequency in IM-resistant CML patients ranged between 2% and 20%.⁽¹³⁻¹⁶⁾ This variability may be related to sensitivities of the technical methods used to detect the mutation^(9,16), the time point of analysis, the treatment response⁽¹⁵⁾, the proportion of patients in primary versus secondary resistance in the different studies. In agreement with previous studies^(17,18), more patients with secondary resistance (10/47, 21.3%) developed T315I mutation during IM treatment than those with primary resistance (4/32, 12.5%).

The best cytogenetic response to IM:-

Cytogenetic response is the gold standard for assessing optimal response and predicting long-term outcome as the primary goal of CML therapy is still the achievement of CCyR.

Ten out of 14 patients with T315I mutation showed an optimal cytogenetic response to IM therapy immediately before the occurrence of resistance or progression and, in concordance with the finding of Norozi et al⁽¹⁵⁾, there was no significant relationship between the mutation status (presence or absence of T315I) and CCyR to treatment.

Time of IM resistance and mutation detection from treatment initiation:-

In our study, most of the T315I+ patients (12/14, 86%) developed IM resistance after one year of treatment. However, the onset of T315I mutations during IM treatment of CML remains challenging. Some authors^(19,20) suggested that mutations may be present at diagnosis prior to therapy, while others⁽²¹⁾ postulated that no instances of mutations develop in early CP before IM initiation and most will develop during treatment. Thus, the presence of T315I mutation remains a time-dependent covariate.

Mutation status and Disease phase at time of IM failure:-

In our study, there was no significant relationship between the mutation status and the phase of the disease at IM failure. This observation might partly be explained by the emerge of additional chromosomal and/or genomic alterations frequently occurred at the time of progression in advanced phase CML in patients with and without T315I mutation as already postulated by different authors.^(22,23)

Among the T315I+ patients, all the 5 patients in BC died and one patient in AP was still alive. This patient developed a sustained CCyR cytogenetic response on second generation TKI. This may support the finding of Jabbour et al.⁽⁹⁾ Eight patients were in CP, suggesting that this mutation is not restrictly confined to patients in advanced stage disease.

On the other hand, 23 out of 24 resistant patients with undetectable T315I mutation in advanced stage (18 patients in BC and 5 patients in AP) died. Therefore, the occurrence of IM resistance and disease progression in T315I negative group of patients may imply the presence of other BCR-ABL mutations (*non-investigated*) or additional genetic abnormalities affecting other unknown BCR-ABL independent mechanisms of IM resistance/progression that provides the mutated clone with a proliferative advantage.⁽²⁴⁻²⁷⁾

Recently, Ng and associates⁽²⁸⁾ identified a novel mechanism of primary resistance to TKIs among *East Asians* compared with other ethnic groups. The TKI resistance was secondary to a germline deletion polymorphism of the BH3 domain of BCL2-like 11 (BIM) that is required for TKIs to induce apoptosis in cancer driven by kinases.

It was noticed that 2 patients in advanced phase (AP) (one patient with and the other without T315I mutation) were escaped from death. Interestingly, these 2 patients received Interferon- α (IFN α) either before IM treatment or after development of resistance. It remains unclear whether IFN- α may induce a protective effect for patients treated with IM⁽¹⁴⁾, or exerts a specific activity on the T315I mutated clone⁽²⁹⁾ or whether it is simply the withdrawal of the TKI, that temporarily improves the outcome of these 2 patients.⁽³⁰⁾ However, Jabbour et al⁽¹³⁾ suggested that there is a correlation between prior IFN therapy and the occurrence of mutations. Thus, the benefit of IFN- α use either before or combining with IM or after development of resistance (as salvage therapy) deserves profound study.

Thirty nine of 79 (49%) resistant patients in CP were still alive due to IM dose escalation or shift to 2nd generation TKI, as early intervention that prevented progression of the disease.

Response to IM dose escalation and second TKI after IM failure:-

In order to overcome resistance, dose escalation of IM (600-800 mg/day) was attempted in 44/79 (56%) of resistant patients. In T315I⁺ patients group, 18 showed good response, 14 patients had their condition stabilized, but for a shorter duration and 6 patients showed no improvement. For T315I⁻ patients, 4 patients in advanced stage showed inadequate response and 2 patients in CP showed good response during 11 and 32 months respectively. Thus, unlike previous studies^(31,32), our study showed that IM dose escalation in patients with T315I mutation could yield, although not sustained, a good response. The good response to dose escalation may support the suggestion of Yamamoto et al⁽³³⁾ that T315I mutation develops IM resistance through increasing the ABL tyrosine kinase activity and dose escalation might overcome this increased activity. Ernst et al⁽³⁴⁾ also reported a good response to therapy with 600 mg IM during 37 months in a CP-CML patient harboring T315I mutation. Thus, these findings are increasingly supporting the suggestion of Nicolini et al⁽¹⁴⁾ that the levels of IM resistance, regardless of the presence or absence of T315I mutation, are heterogeneous, ranging from slightly reduced sensitivity to IM (overcome by IM dose escalation) to absolute insensitivity to IM (requiring other treatment modality).

Among the 14 patients with T315I, 8 patients were shifted to second generation TKI (Dasatinib) after failure of IM treatment. Responses were observed in 3 patients (Sustained CHR, sustained CHR/CCyR, sustained PHR/transient minor CyR). This finding did not match with most of previous studies,⁽³⁵⁻³⁸⁾ which showed that resistant CML patients bearing T315I mutation do not respond to second generation TKI, our finding in this respect may support that of Jabbour et al⁽⁹⁾ who first reported sustained responses of T315I-bearing CML clones to second TKI, indicating that this mutation does not predict for resistance in all cases.

The presence of controversy in the responses to IM dose escalation and 2nd generation TKI in some of our T315I+ IM-resistant patients from most of the previous studies might be – at least partially- related to the mutation pattern heterogeneity and to the geographical and ethnical differences of the studied population as suggested by Ng et al⁽²⁸⁾ and Nicolini et al⁽³⁹⁾ in their study which included patients from 9 countries (from Europe, Asia and North America).

Impact of mutational status, disease phase and response duration on OS:-

Although *in vitro* studies⁽⁴⁰⁾ demonstrate that the BCR-ABL T315I mutation provides the mutated clone with a proliferative capacity over BCR-ABL WT cells and favors disease transformation, our study showed no difference in survival between patients with, and without T315I, in concordance with the previous studies of Jabbour et al^(9,13) who concluded that neither the presence of mutations nor the type of mutation was associated with survival among patients who failed IM. On the contrary, other studies^(14,35,41) suggested that the presence of T315I mutation is associated with significantly poor prognosis and reduced overall survival. Yet, it remains unclear whether the presence of T315I mutation is actually responsible for disease progression through disruption of IM binding or interacts with other general factors of progression such as cytogenetic clonal evolution or additional mutations or it is simply present in a cell that is resistant to IM for other unknown reasons. Therefore, chromosomal abnormalities other than the Ph¹, and additional mutation at T315I identification should be evaluated to know if there are other mechanisms of resistance. Thus, the impact of T315I mutation on survival depends on several factors.⁽¹³⁾ In addition, the extent of resistance of IM and other TKIs to T315I mutation may vary greatly⁽⁴²⁾ and it remains unclear why some patients developed IM-resistance with detectable levels of T315I clones while others do not.⁽⁴³⁾

Our study demonstrated also that survival is mostly dependent on the duration of IM response and the disease phase at the time of IM failure. *Shorter response duration* and *advanced stage* disease were associated with poor survival.

On the other hand, the above results indicated no significant relationship between T315I mutation, duration of response to treatment, CCyR to treatment, blood parameters and patients' clinical signs. Therefore, the evaluation of this mutation is recommended in all the patients in different phases of disease, even with a favorable response to therapy, which may be associated with relapse of their disease in the future and requires comprehensive studies in a longer period of time.⁽¹⁵⁾

Conclusion:-

Therefore, it had be concluded that the detection of T315T mutation in BCR-ABL-positive Egyptian resistant patients has nothing to do with patient survival, treatment period and CCyR and the survival of these patients remains largely dependent on the disease phase at the time IM failure, as well as the duration of response to IM therapy.

Lastly, the fact that we did not observe clear treatment pattern after T315I mutation detection highlights the need for evaluation of chromosomal abnormalities other than the Ph¹, and additional mutation at T315I identification to know if there are other mechanisms of resistance.

Conflict of Interest:-

The authors declare no conflicts of interest in preparing this article.

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