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# Detection of a Point Mutation at Codon 12 of the Kirsten-Ras (K-ras) Oncogen in Myelodysplastic Syndrome

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## Abstract

**Background:** Mutations in ras genes have been observed in a variety of cancers and were found to play an important role in human leukemogenesis and in preleukemic disease as myelodysplastic syndrome (MDS). The purpose of this study was to determine the prevalence of mutated K-ras oncogene in myelodysplastic syndrome (MDS); with a special emphasis on their possible role in affecting clinical status, relation to karyotypic pattern; response to therapeutic measures; its impact on the fate of the disease and overall survival.

**Subjects & methods**: Detection of point mutation of Kirsten-ras (K-ras) gene in 30 patients suffering from myelodysplastic syndrome was carried out using quantitative enriched polymerase chain reaction (QEPCR) and was confirmed by sequencing. QEPCR is a two- stage PCR procedure with modified primers that enriches mutant alleles, via restriction endonuclease digestion of normal alleles and enables identification of one mutant allele among 100,000 normal alleles.

**Results:** Activating mutations of the codon 12 of K-ras gene were detected in 7/30 (23.3%)cases of MDS, the most common mutation involved a substitution of aspartic acid for glycine (GGT→GAT). The incidence of K-ras mutations was found to be significantly associated with refractory anemia with excess blasts type II (RAEBII) and unclassified (UC) MDS than other subtypes (p=0.005), and was significantly associated with hypercellular bone marrow (p=0.04) showing marked dyserythropoitic changes. Furthermore, mutant K-ras gene was found to be significantly associated with abnormal karyotypes (p=0.04). Patients with mutated K-ras gene were significantly associated with either high or intermediate risk according to International Prognostic Scoring System (IPSS) (p=0.001). 6/7(85.7%) of those carrying the mutation showed poor response to treatment compared to non carriers with a statistical significant difference (p=0.009). Five out of eight (62.5%) patients who were transformed to AML carried the mutant K-ras gene, their subtypes were RAEBII and unclassified MDS with abnormal cytogenetics mainly Monosomy 7. Overall survival was detected using Kaplan-Meier curve and the mean survival time of patients who carried K-ras mutations were significantly lower than those without the mutation (Log rank test=12.7; p=0.0004).

**Conclusion:** MDS patients bearing an mutated K-ras oncogene frequently showed poor response to treatment; leukemic progression of the disease and shorter overall survival, suggesting that an activated K-ras oncogene is a critical factor for prognostic evaluation; therapeutic decision and monitoring of response to treatment of MDS patients.

**Key words**: K-ras, QEPCR, MDS.

## Introduction

Ras genes encode a family of 21-kd proteins (H-ras, K-ras, and N-ras)( **Liu *et al.*, 1987)** They play a role in signal transduction thus regulating cell proliferation, differentiation, and survival **(Lièvre *et al.*, 2008)** Mutations in these genes usually result from single amino acid substitutions at one of the

critical regions of the protein. These mutations have been detected in codon 12 or 13 of the ras gene **(Amado *et al*., 2008)** They are activating mutations and have been observed in a variety of cancers resulting in continual signal transduction and stimulation of downstream signaling pathways involved in cell growth, proliferation, invasion, and metastasis **(Eberhard *et al., 2005)***

Myelodysplastic syndromes (MDS) are clinically heterogenous disorders characterized by clonal hematopoiesis, impaired differentiation, peripheral blood cytopenias and a risk of progression to acute myloid leukemia (AML) **(Ohara *et al*.,2002)** Somatic mutations may influence its clinical phenotype and overall survival**(Ahuja et *al*.,1990& Bejar *et al*., 2011)**An understanding of the effects of mutations in various genes as ras genes could improve the prediction of prognosis in these patients and inform for the selection of specific therapies **(Nakagawa *et al*.,1992 & Sekeres ,2010)**.

In view of such data, the purpose of this study was to determine the prevalence of activated K-ras oncogene in MDS patients; with a special emphasis on their possible role in affecting clinical status, response to therapeutic measures; impact on the fate of the disease and overall survival.

## Subjects and Methods:

This study included 30 MDS patients attending Hematology Unit, Ain Shams University and Wadi El-Neel Hospitals from September 2007 to September 2010. They were 19 males and 11 females with a ratio of 1.7:1. Their age ranged from 21-67 years with a mean age of 42 years. The diagnosis was based on standard clinical and haematological criteria using histopathology, cytochemistry and cytogenetic analysis**(Nosslinger *et al*., 2000).**

All patients were subjected to the following:

-Thorough history taking and clinical examination laying stress on hepatomegaly, splenomegaly and lymphadenopathy.
-Complete blood counts using Coulter Gen S (Coulter Electronics,USA) and leishman stained blood smears.
-Bone marrow (BM) aspiration and trephine biopsy examination
-Conventional Cytogenetic analysis (CCA) by G-banding (**Haase *et al*., 2007**).
**-**Mutant K-ras codon 12 alleles detection by Quantitative Enriched Polymerase chain reaction (QEPCR) followed by DNA sequencing **(Ronai and Minamoto,1997).**

MDS patients were divided into 3 risk groups according to International Prognostic Scoring System (IPSS) criteria**(Garcia-Manero *et al.*, 2008)**as regards BM blasts % (<5%, 5-10%, 11-19% &>20%), Cytopenias (Hb<10g/dl, neutrophils$<1.5\times10^9$/L and platelet$<100\times10^9$/L) (**Ohara *et al*.,2002** ) and Karyotype pattern (low risk patients with normal karyotype, chromosome y deletion, del (20q),del (5q)); high risk patients with chromosome 7 anomalies or complex karyotype (3 abnormalities) and intermediate risk patients with any other anomalies as trisomy 8, single or double abnormality).

Treatment strategy was by: Supportive treatment: (irradiated packed RBCs with symptomatic anemia or Hb<8g/dL; platelets concentrates with active bleeding or platelet count$<10x10^9$/L)**;** Hemopoietic growth factor and folic acid; others (immunotherapy, chemotherapy or combined), Bone marrow and stem cell transplantation.

Patients were followed up for 36 months for evaluation of: treatment response by repeated complete blood counts and bone marrow aspirate, complete response and relapse were defined according to standard criteria (**List,2008**). Assessment

of overall survival (OS) was analyzed by Kaplan-Meier curves and Log Rank test. Overall survival was measured from day of diagnosis until death from any cause, or patients known to be alive at last contact.

**Sampling**

For detection of K-ras mutation by QEPCR: Five milliliters bone marrow or 5-10 ml peripheral blood was collected on EDTA. For cytogenetic analysis: 1ml BM in sterile, preservative-free-heparin, coated vacutainer tube. Samples were collected after informed consent.

## Methods

**Conventional cytogenetic analysis (CGA) by G-banding** :

This analysis involves the examination of spontaneously dividing cell populations by blocking cell division at metaphase stage with an inhibitor of spindle formation (colcemid). This is followed by hypotonic wash and fixation then slide making and staining with Giemsa stain using trypsin to induce G-banding. Analysis of available metaphase was performed using light microscope and image system.

**Mutant K-ras codon 12 allele detection:**

*Quantitative Enriched Polymerase chain reaction (QEPCR)*

QEPCR is two steps PCR procedure involving an initial PCR of K-ras target, restriction digest of wild type sequences followed by a second PCR that amplifies remaining mutant target sequence.

-DNA extraction: Genomic DNA was isolated using QIAamp DNA Minikit (Qiagen, Germany) according to manufacturer's instructions and stored at -80°C until used.

-PCR amplification: Fragment encompassing exon 1 of human K-ras gene was amplified by QEPCR. Primers used for first round PCR were: Forward primer: 5'-GCG GTT GGG GCT TAA TTG CAT ATA AAC TGA ATA TAA ACT TGT GGT AGT TGG ACCT-3'.
Reverse primer: 5'-GCT GTT GTC ATA GTA ATG ATC TCA TTC CAV TGT ACT CCT C-3'.
Primers used for 2nd round PCR were: Forward primer: 5'-GCG GTT GGG GCT TAA TTGCA-3'.
Reverse primer: 5'-GCT GTT GTC ATA GTA ATG AT-3'.

During the first round of amplification the reaction was carried out in a total volume of 50 ul containing 1 ug of genomic DNA, 0.12pmol/ul of each primer, 0.2mM of each dNTPs, 0.5 unit thermostable Taq DNA polymerase and 1X Reaction buffer (all reagents were supplied by Promega, USA). Cycling consisted of 20 cycles: denaturation 94°C for 1 minute, annealing 59°C for 1 minute, extension 72°C for 1 minute and finally another 5 minutes at 72°C. 5 ul of amplified material was used for restriction enzyme digestion using MVaI (12 units) supplied by (Boehringer Mannheim Biochemica) and respective buffer in total volume of 20 ul for 2 hours at 37°C.

From the digested material 5 ul was taken for the second amplification using 0.12 pmol/ul of each primer of second round. Other reaction and cycling conditions were as for the first round. 2% Agrose gel electrophoresis was used to confirm the product of amplification with 190 bp for the mutant gene, 146 bp for the wild type. Normal samples were included in each run (Fig1).

*DNA Sequencing of mutated cases*

Product from the second PCR was used for DNA sequencing. Mutated samples were sequenced with the primers used in the second QEPCR reaction. Sequencing with a dye terminator cycle sequencing kit (Applied Biosystems,UK) following the manufacture's instruction. The sequences were

analyzed on an ABI PRISM 310 genetic analyzer, Perkin Elmer (Applied Biosystems, UK). (Fig1).

### Statistical analysis

It was performed by using SPSS 9 statistical software on IBM compatible PC. Continuous data parameters were described as mean ± SD. For comparative study, chi- square ($X^2$) test for non parametric data and Student t (t) test for parametric data were used. Survival analysis was performed using Kaplan Meier curves and Log Rank test. The probability (p value) was considered significant if $p<0.05$.

### Results

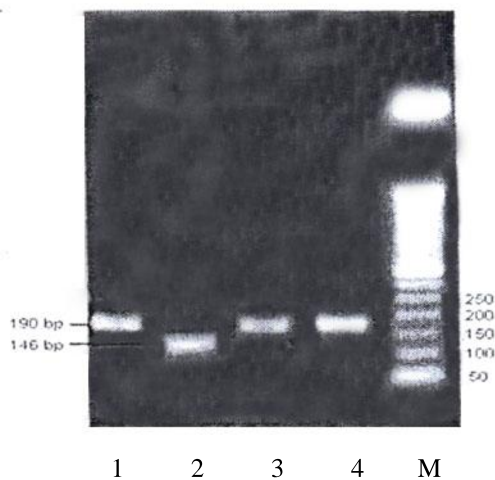
Demographic , clinical characteristics and laboratory data of the study patients are detailed in tables 1 & 2. Clinically 36.7% had organomegaly while 13.3% had lymphadenopathy.

Peripheral blood picture showed that 50% of the patients suffered of pancytopenia while 43.3% were bicytopenic and 2 patients had anemia only. Bone marrow was hypercellular in 40% of cases, hypocellular in 26.7% and normocellular in 33.3%. The BM examination showed dyserythropoiesis in 63.3%, dysgranulopoiesis in 33.3% and dysmegakaryopoiesis in 16.7 %. According to WHO classification; refractory anemia (RA) was diagnosed in 53.3% of patients; refractory anemia with ringed sideroblasts (RARS) in 6.7%; refractory anemia with excess blasts type I (RAEBI) in 10%, refractory anemia with excess blasts type II (RAEBII) in 20%, refractory cytopenia with multiple dysplasia (RCMD) in 6.7%, unclassified (UC) MDS in 3.3% (Table1). Cytogenetic analysis showed a normal karyotype in 18 (60%) of patients, whereas chromosomal abnormalities were observed in 12 (40%) patients. The most frequent karyotype was monosomy 7 which was observed in 4/12 (33.3%) cases. 5q- was detected in 2 patients; trisomy 8 was in 2 patients; trisomy 21 was in 1 case; del 17 in 1 case while 2 cases showed complex karyotype.

IPSS risk classification had been ascertained at the time of diagnosis. Accordingly, our MDS patients were classified into 3 prognostic groups: 46.7% (14 patients) were low risk most of them had RA with normal karyotype. 36.7% (11cases) showed intermediate risk with various MDS subtypes, 7 of which showed abnormal karyotypes: 2 had trisomy 8, 1 case had trisomy 21, 1 case had del 17, 2 cases had 5q-and 1 had complex karyotype (combined del 3 & del 6). Five (16.7%) cases were classified as high-risk. They carried chromosomal abnormalities (monosomy 7 & complex karyotype) and were associated with RAEBII and unclassified MDS.

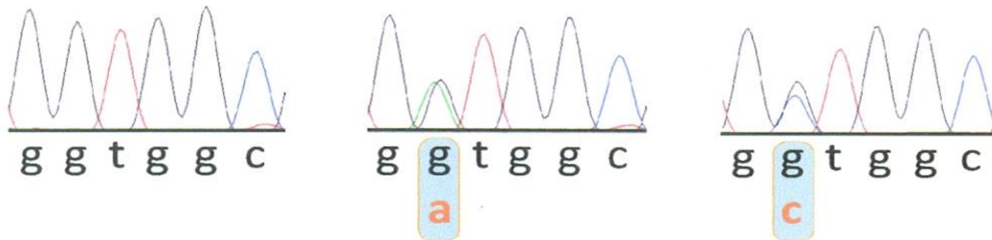
Of the 30 MDS patients included in this study, seven (23.3%) were positive for point mutations of the K-ras gene at exon 1, codon 12. All mutated cases detected by QEPCR were confirmed by DNA sequencing. The nature of the point mutations involved a substitution of aspartic acid for glycine (GGT→GAT) in 6 patients. Only one patient showed a substitution of argenine for glycine (GGT→GCT). Normal samples were included in each run and were negative for K-ras mutations. According to presence and absence of the mutation, the clinical characteristics and laboratory data of patients were compared**.** The incidence of K-ras mutations was found to be significantly associated with RAEBII and UC subtypes ($p=0.005$) and was associated with bone marrow hypercellularity ($p=0.04$), moreover 85.7% of patients with mutant K-ras showed marked dyserythropoitic changes. Again 71.4% of patients carry K-ras mutation were found to have abnormal karyotype with a statistical significant difference ($p=0.04$). They were significantly associated with high and intermediate risk classification according to IPSS ($p=0.001$). Moreover they were significantly associated with poor response to treatment (($p=0.009$;Table1). Patients with K-ras mutations were more likely to have elevated blast percentage with a statistical significant difference ($t=2.89$;$p=0.007$), but the two groups did not differ significantly with respect to leucopenia, anemia or thrombocytopenia ($p>0.05$; Table2). On following up of the patients, 63.3% showed favorable response to treatment (complete remission or stable disease) while 36.7% showed unfavorable response (incomplete remission, relapse or treatment failure). Presence of K-ras was significantly associated with unfavorable response to treatment as 85.7% (6/7) of

those carrying the mutation showed unfavorable response to therapy (p=0.009; Table 1).
Moreover, 8 (26.7%) of MDS patients evolved to AML (BM blasts>20%). It occurred mainly in patients having hypercellular marrow. 5/8 (62.5%) of them carried mutated K-ras gene, with RAEBII and unclassified MDS. By the end of the study, 70% of cases lived and 30% died. K-ras mutation was statistically associated with the fate of the patients as 5 of the 7 (71.4%) cases carrying the K-ras mutation died ($X^2$= 5.96; p=0.01) and the risk estimation for the presence of K-ras mutation showed an odd ratio of 1.8. Kaplan-Meier analysis of overall survival showed that the mean survival of patients with non-mutated K-ras gene was 23.4 months with 95% CI (20.1-26.8) while patients with mutated K-ras showed a mean survival of 14.8 months with 95% CI (9.5-17.5) and a Log Rank test of 14.7 showing a high statistical significant difference (p=0.0001;Figure2).




1 2 3 4 M

**Photo1: Electrophoretic separation of PCR amplification product**
Lanes 1,3,4: mutant type of K-ras
Lane 2: wild type of K-ras. M: molecular marker




(a) (b) (c)

**Fig.1: DNA sequence analysis for K-ras gene at codon 12**
(a) wild type of K-ras. (b) G to A mutation. (c) G to C mutation.

**Table (1): Patients characteristics according to presence or absence of K-ras**

| Parameters (n=30) | K-ras | | $X^2$ | p |
|---|---|---|---|---|
| | Non-mutant(23) n(%) | Mutant(7) n(%) | | |
| **Sex** | | | | |
| Male (19) | 13(56.5) | 6(85.7) | 1.96 | 0.215 |
| Female (11) | 10(43.5) | 1(14.3) | | |
| **Organomegaly** | | | | |
| -ve (19) | 15(65.2) | 4(57.1) | 0.15 | 0.65 |
| +ve (11) | 8(34.8) | 3(42.9) | | |
| **Lymphadenopathy** | | | | |
| -ve (26) | 20(87) | 6(85.7) | 0.01 | 0.93 |
| +ve (4) | 3(13) | 1(14.3) | | |
| **Cytopenia** | | | | |
| Anemia (2) | 2(8.7) | - | 1.906 | 0.38 |
| Bicytopenia (13) | 11(47.8) | 2(28.6) | | |
| Pancytopenia (15) | 10(43.5) | 5(71.4) | | |
| **MDS type** | | | | |
| RA (16) | 16(69.5) | - | 16.9 | 0.005* |
| RARS (2) | 1(4.3) | 1(14.3) | | |
| RAEB I (3) | 3(13) | - | | |
| RAEB II (6) | 2(8.6) | 4(57.1) | | |
| RCMD (2) | 1(4.3) | 1(14.3) | | |
| UC (1) | - | 1(14.3) | | |
| **BM celularity** | | | | |
| Normo (10) | 10(43.5) | - | 6.05 | 0.04* |
| Hypocellular (8) | 7(30.4) | 2(28.6) | | |
| Hypercellular (12) | 6(26.1) | 5(71.4) | | |
| **Cytogenetics** | | | | |
| Normal (18) | 16(69.6) | 2(28.6) | 3.89 | 0.04* |
| Abnormal (12) | 7(30.4) | 5(71.4) | | |
| **IPSS Risk** | | | | |
| Low (14) | 14(60.9) | - | 13.3 | 0.001* |
| Intermediate (11) | 8(34.8) | 3(42.9) | | |
| High (5) | 1(4.3) | 4(57.1) | | |
| **Response to ttt** | | | | |
| Favorable (19) | 18(78.3) | 1(14.3) | 6.904 | 0.009* |
| Unfavorable (11) | 5(21.7) | 6(85.7) | | |

RA:refractory anemia. RAEB:refractory anemia with excess blasts. RARS:refractory anemia with ringed sideroblasts. RCMD: refractory cytopenia with multiple dysplasia. UC: unclassified. IPSS:International Prognostic Scoring System; ttt:treatment.*Significance level p<0.05.

**Table (2): Comparison between laboratory results according to presence or absence of K-ras**

| Parameters (n=30) | K-ras | | t | p |
|---|---|---|---|---|
| | Non-mutant (mean±SD) | Mutant (mean±SD) | | |
| Age (y) | 40 ± 2.2 | 47 ± 6.5 | 1.28 | 0.21 |
| TLC ($x10^9$/L) | 5.1 ± 1.3 | 3.3 ± 1.5 | 1.02 | 0.91 |
| Hb (gm/dl) | 8.1 ± 1.9 | 8.2 ± 0.7 | 0.013 | 0.98 |
| Platelets ($x10^9$/L) | 87.3 ± 15.4 | 101.7±46 | 0.38 | 0.704 |
| BMblast (%) | 5.9 ± 3.2 | 30.2±11.2 | 2.89 | 0.007* |

SD:Standard deviation; TLC: total leucocytic count; Hb:hemoglobin; BM:bone marrow *Significance level p<0.05.

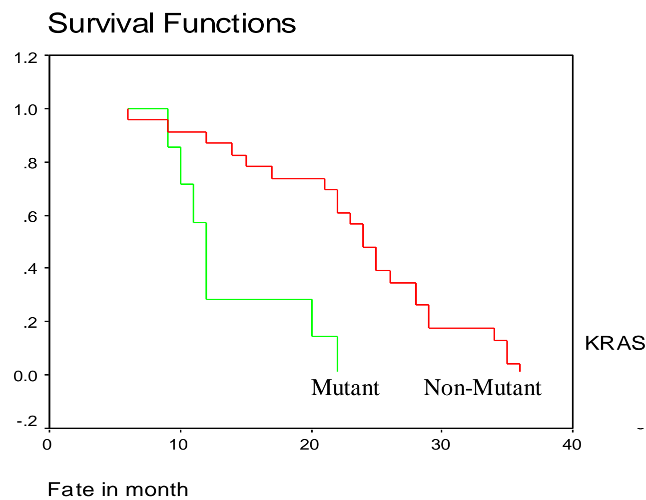



**Figure (2): Kaplan–Meier analysis of overall survival according to presence or absence of K-ras**; (Log Rank=12.7, p=0.0004).

## Discussion

K-ras oncogenes activated by point mutation have been frequently detected in various types of human cancers **(Rocquain *et al.*, 2010 ) .**Analysis of a large number of leukemias revealed that activated K-ras oncogenes were observed preferentially in AML, T-ALL and Null-ALL. These results suggest that K-ras oncogenes play an important role in human leukemogenesis **(Liu *et al.*, 1987 ).**Activated K-ras oncogenes were also detected in myelodysplastic syndrome (MDS) which is considered to be a preleukemic disease **(Rocquain *et al.*, 2010 ).**

In the present study the K-ras point mutation in exon 1, codon 12 was detected in 23.3% of cases, in close agreement to that reported previously **(Harada &Harada ,2011 )** but differs from those reported by other researchers who showed different frequencies ranging from 0% up to 40%. This discrepancy may reflect the variable sensitivities and specificities of different techniques used or may reflect heterogenity in the patient populations studied **(Ronai & Minamoto ,1997).**

Several investigators have suggested that there is a heterogeneity of leukemic cells with respect to the presence of activated ras gene and that in some patients only a fraction of malignant cells carry the mutant gene **(Farr *et al.*,1988 )**this was explained by

that the mutation occurs later after a preleukemic clone has already emerged thus giving the premalignant clone an additional growth advantage. An alternative explanation was that the ras mutation occurred early in the preleukemic process and that there was later evolution with the emergence of a clone which has been activated by another gene **(Nakagawa *et al*., 1992).**

Furthermore, in this report the most common mutation was involving a substitution of aspartic acid for glycine (GGT→GAT), this was in accordance to a previous report**(Liu *et al*.,1987)** which explained that this may reflect either involvement of a specific unknown mutagen or selective advantage offered in vivo by this mutation in the ras protein (**Bejar *et al*.,2011**).

In this study, K-ras mutation strongly associates with RAEBII subtype and with BM hypercellularity. Again, our results showed that 85.7% of patients with positive K-ras suffered of marked dyserythropoitic changes this finding is consistent with and support the notion that mutant K-ras directly impairs erythropoiesis in patients with MDS, as transduction of human erythroid progenitors with oncogenic ras results in hyperproliferation but with defective differentiation resulting in reduced production of mature erythrocytes **(Nakagawa *et al*., 1992** ).

In the current study, patients with K-ras mutations were more likely to have elevated blast percentage than the non mutant cases with a significant difference (p=0.007), but the two groups did not differ significantly with respect to leucopenia, anemia or thrombocytopenia as previously reported (**Ahuja *et al*.,1990**).

Again in this report, K-ras mutation was found to be significantly associated with abnormal karyotypes as 71.4% of patients carrying the mutation showed abnormal karyotypes; moreover the presence of the mutation was strongly associated with high risk (p=0.001). Thus denoting the importance of K-ras mutation in MDS and its prognostic significance **(Bejar *et al*., 2011).**

In the present report, following up, of cases showed favorable response in 63.3% while 36.7% showed failure of treatment. 85.7% of those carrying the mutation showed poor response to treatment as previously reported **(List ,2008 )** showing that K-ras mutations at exon 1 codon 12 have been associated with lack of response to therapeutic agents targeted to signaling pathways which are regulated by K-ras protein, thus recommending to test K-ras mutation before initiating these therapies furthermore alternative therapies should be considered for patients with K-ras mutations **(Linardou *et al*.,2008).**

In this report, 8 patients transformed to AML, 5 of them carried the mutant K-ras gene, and their subtypes were RAEBII and unclassified MDS. Additionally, their previous bone marrow examination showed mainly hypercellularity. This is in agreement with previous studies **(Amado *et al*., 2008 & Harada and Harada 2011)** showing that MDS patients with mutations may evolve to AML more frequently than those without mutations. Conversely, other observers found reduction of mutant K-ras gene with leukemic transformation **(Kosmider *et al*., 2010)**. Furthermore, the existence of ras mutations in diverse myeloid malignancies raises the possibility that they invariably represent secondary events that cooperate with a spectrum of initiating genetic lesions. Consistent with this possibility, it was found that some AMLs contain subclones with independent ras mutations moreover other ras mutations that are detected at diagnosis may disappear over time in patients

with persistent or relapsed disease **(Malcovati *et al*., 2007& Bejar *et al*.,2011)**. Other investigators found that patients who progressed to AML without K-ras mutation may carry transforming gene other than K-ras **(Ahuja *et al*., 1990 ).** Thus it was proposed that the presence of K-ras gene mutation may be of prognostic value. However there are other factors which are important in prognosis. Also, in the present study we found that hyperplastic marrow had a significantly high frequency of progress to AML as previously reported **(Sakuma *et al*., 2006 ).** Moreover, our results showed that RA and RARS typically have the lowest incidence of leukemic evolution, whereas RAEB is the highest, this is in accordance to a previous study **(Pardanani *et al*.,2010 ).**

By the end of this work, 70% of patients lived while 30% died. 5 of the 7 (71.4%) cases carrying the K-ras mutation died. Survival analysis confirmed the prognostic validity of K-ras, as the risk estimate for K-ras showed high odd ratio of (1.8).

Kaplan-Meier analysis of overall survival showed the mean survival of patients with non-mutated K-ras was 23.4 months compared to14.8 months months in patients with mutated K-ras showing a high statistical significant difference (p=0.0001). Similar results were obtained previously **(Bejar *et al*., 2011 ).**

In conclusion, MDS patients bearing a mutated K-ras oncogene frequently showed poor response to treatment; leukemic progression of the disease and shorter overall survival, suggesting that an activated K-ras oncogene is a critical factor for prognostic evaluation; therapeutic decision and monitoring of response to treatment of MDS patients.

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# تقييم التحول الجينى للK-ras فى رامزة 12 لمرضى متلازمة سوء النمو النخاعي

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**المقدمة:**
لوحظ وجود التحول الجينى لل K-ras فى عدد كبير من الأورام . و وجد أنه يلعب دور فعال فى إبيضاض الدم و فى مقدمة الإبيضاض مثل متلازمة سوء النمو النخاعي. و قد هدف هذا البحث إلى تقييم إنتشار التحول الجينى لل K-ras ل30 من مرضى متلازمة سوء النمو النخاعي مع إظهار دوره المحتمل فى التأثير على الحالة الإكلينيكية و علاقته بالتركيب الجينى للمرضى والإستجابة للوسائل العلاجية و كذلك تأثيره على مآل المرض . و قد تم تحليل التحول الجينى لل K-ras بطريقة (QEPCR) التي تمكن من تحديد نظير واحد متحور لل K-ras من وسط 100,000نظير طبيعى وقد تم التأكد من النتائج بإستخدام تقنية تتابع الحمض النووى.

**النتائج:**
وقد أظهرت النتائج وجود التحول الجينى لل K-ras فى 23.3 % من المرضى وكان إحلال Aspartic acid لل Glycine هو التحور الأكثر شيوعا.وقد وجد أن هذا الخلل الجينى كان الأكثر شيوعا مع الأنيميا الغير مستجيبة للعلاج و التى فيها زيادة فى الخلايا السرطانية (RAEBII) و كذلك الغير مصنفة و التى يصحبها زيادة ملحوظة فى عدد خلايا النخاع الشوكى. كما أنه وجد علاقة بين وجوده و بين وجود الفحص الوراثى الغير طبيعى. و وجدت علاقة قوية بين التحول الجينى لل K-ras وشدة خطورة المرض حيث أن كل المرضى حاملى التحول تم تقسيمهم إلى متوسطى وأعلى خطورة للمرض و كذلك كان له علاقة ذات دلالة إحصائية مع عدم الإستجابة الجيدة للعلاج.كذلك وجد أن 62.5% من المرضى حاملى التحول الجينى لل K-ras قد تحولوا إلى سرطان دم ميلودى الحاد. و قد وجد أن أكثرهم يحملون الخلل الكروموزومى 7 الاحادى.وقد تم تقييم العلاقة بين النمط الجينى لل K-ras ومآل المرض حيث وجد أن مرضى متلازمة سوء النمو النخاعى حاملى التحول الجينى كانوا أقل فى متوسط زمن البقاء بالمقارنة مع غير الحاملين لهذا التحول الجينى مع وجود فرق ذو دلالة إحصائية.

**الإستنتاج:**
وقد خلص هذا البحث إلى أن مرضى متلازمة سوء النمو النخاعى الذين يحملون التحول الجينى لل K-ras فى أغلب الأحيان يظهرون إستجابة ضعيفة للعلاج مع التطورلإبيضاض الدم الحاد وتقلص فترة الحياة موحيا إلى إعتباره عامل حرج للتقييم المنذروللقرار العلاجى وكذلك لمتابعة الإستجابة للعلاج فى هؤلاء المرضى.