ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 8, Issue, 9, pp. 19759-19765, September, 2017

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

ASSESSMENT OF CYTOGENETIC CHANGES AND EXPRESSION OF LET-7A MICRORNA IN RELATION TO ITS TARGET GENE NRAS IN MYELODYSPLASTIC SYNDROME (MDS)

Elahe ghods[1]., Zahra Mozaheb[2]*., Shermineh Heydari[1]., Mohammad Hasanzadeh-Nazarabadi[1]., Majid Mojarad[1] and Ehsan Ghayoor Karimiani[3]

[1]Medical Genetic Department, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[2]Hematology-Oncology Department, Mashhad University of Medical Sciences, Mashhad, Iran
[3]Molecular Diagnostic unit, Research and Education Department, Razavi Hospital, Mashhad, Iran

DOI: http://dx.doi.org/10.24327/ijrsr.2017.0809.0754

ARTICLE INFO

Article History:

Received 18th June, 2017
Received in revised form 10th July, 2017
Accepted 06th August, 2017
Published online 28th September, 2017

Key Words:

Myelodysplastic syndrome, Cytogenetic, miRNA let-7a, NRAS

ABSTRACT

Background: Myelodysplastic syndromes (MDSs) are a heterogeneous group of disorders characterized by clonal disorder and ineffective hematopoiesis. Cytogenetic abnormalities can be detected in approximately 50% of patients with de novo MDS and are one of the main keys of prognostic factor. On the other hand, miRNAs that regulate gene expression of complementary mRNAs are crucial key of cellular processes which their deregulation is implicated in the disease development and progression. The purpose of this study is to assess cytogenetic and expression of miRNA let-7a and its target gene NRAS in order to find whether any correlation between IPSS based on cytogenetic and expression of this microRNA and gene in patients does exist.

Method: In this study the 21 patients with de novo MDS between were selected, and 25 control groups were healthy people. Chromosomal analysis of lymphocyte cultures were performed on the basis of G-banding technique at high resolution. For molecular analysis: RNA extraction, cDNA synthesize, survey expression of microRNA by real-time quantification PCR were applied.

Results: Twenty patients appear with a normal karyotype whereas only one patient revealed the constitutive heterochromatic region below the centromeres of chromosomes 1 (46,XX(1qh+)). The expression of the Let-7a miRNA was decreased whereas its target NRAS gene was increased in our patients as compared with the controls group (P value <0.0001).

Conclusion: According to decreasing expression of miRNA let-7a and increasing expression of NRAS in MDS patients, we suggest, using miRNA, molecular and cytogenetic panel as a prognostic marker in MDS patients. Future studies are needed to confirm it.

INTRODUCTION

Myelodysplastic syndromes (MDSs) are a heterogeneous group of hematopoietic stem cell disease characterized by cytopenia, dysplastic-appearing bone marrow and ineffective hematopoiesis (Nimer, 2008; Malcovati *et al*., 2013; Vasilatou *et al*., 2013b). The incidence rate of MDS is approximately 5 cases per 100 000 persons per year before age 60 years, but this rate will increase to 20-50 cases per 100 000 persons per year in people over 60 years (Mufti *et al*., 2008; Malcovati *et al*., 2013). Male to female ratio 1:1 has been reported. The most common form of MDS is sporadic while familial MDS is rare (Owen *et al*., 2008; Nickels *et al*., 2013; Heydari *et al*., 2016). The clinical phenotypes of patients are different including the number and degree of peripheral cytopenias, bone marrow cellularity and blast count, genetic abnormalities, and overall survival and rate of leukemic transformation, as well as response to different therapies (Germing *et al*., 2008; Rainer and Ulrich, 2014). Chromosomal structure changes are the most common cytogenetic abnormalities in MDS (Haase *et al*., 2007; Mufti *et al*., 2008; Rhyasen and Starczynowski, 2012). In addition to FAB and WHO classification which based on the morphological criteria, a new international Prognostic Scoring System (IPSS) permit to assess the patients' prognosis and the treatment decision-making in MDS. In recent years, a major effort from several MDS cooperating groups has led to the development of the Revised IPSS (IPSS-R), based on the new cytogenetic scoring system. IPSS-R better defined the role of cytogenetics as a major MDS prognostic determinant, with 16

Corresponding author:* **Zahra Mozaheb
Hematology-Oncology Department, Mashhad University of Medical Sciences, Mashhad, Iran

specific abnormalities (Greenberg *et al*., 2012; Voso *et al*., 2013; Rainer and Ulrich, 2014). The International Prognostic Scoring System (IPSS), has a limited ability to predict MDS patient outcomes, in addition cytogenetic abnormalities only can be seen in 50% of patients with MDS, therefore; molecular markers are needed to improve prediction accuracy (Erdogan *et al*., 2011; Zuo *et al*., 2011).

MicroRNAs are small (19-24 nucleotides), non-coding RNAs which are responsible for gene expression at the post-transcriptional stage. MiRNA expression profiles have potential as specific and noninvasive biomarkers of cancer; especially in MDS could improve diagnosis, prognosis and disease management (Zuo *et al*., 2011; Vasilatou *et al*., 2013b). In current study, we decided to investigate patients's cytogenetic with MDS in order to find Relationship between chromosomal disorder and this disease. Another goal of the present study was evaluation of expression of miRNA let-7a and its target NRAS in plasma samples from MDS patients compared with controls group to assess their potential clinical significance and prognostic factor.

MATERIALS AND METHODS

Patients and control group

Peripheral blood samples from 21 with *de novo* MDS were collected by considering the hematologic disorders as well as clinical features and other parameters who were seen at the University of Mashhad Emam Reza Hospital between 2014 and 2015. The control group were the 25 healthy individuals with 40-90 years old and normal karyotype, absence any type of infection and Hematological disorders at the time of study. Patients were excluded if they were younger than 40 years and had therapy associated MDS, missing cytogenetic data or an unsuccessful cytogenetic examination. Written informed consent was obtained from all participants. Moreover, this research project has been approved by the ethics committee of Mashhad University of Medical Sciences. The MDS patients had a median age of 65 years (range, 45-85 years) and a male-to-female ratio of 2:1. Since the incidence of MDS increases with age, suitable control samples were chosen; 25 control groups were healthy people between 40 to 90 years old. MDS patients had no known history of previous cytotoxic therapy.

Approximately 6 mL of peripheral blood was obtained from each participant and divided in two tubes with heparin, for cytogenetic analysis, and EDTA, for molecular analysis.

Cytogenetic

Heparinized blood was cultured by two methods: routine culture and High resolution (HR) culture.

Both of the cultures were performed as follow: 0.5 mL peripheral blood was incubated at 37°C with 5 mL RPMI 1640 (Gibco BRL, USA) cell culture medium supplemented with 20% fetal bovine serum (FBS) (Gibco BRL) and 0.1 mL phytohemmaglutinin (PHA).

After 48 hours incubation period, 100µL thymidine was added to each HR culture tube and again they were incubated at 37°C for 16-18 hours. After that, thymidine should be removed from culture with 5 mL sterile PBS (thymidine wash). The tubes were incubated with culture free PHA for about four hours. Culture tubes were harvested by 50µL colcemid (Gibco BRL) and incubated at 37°C for 15 min. The content of the tubes were then centrifuged for 10 min at 1700 rpm and resuspended treated in hypotonic solution (10 mL 4.5 g/L KCl (Sigma, Co)) and were incubate at 37°C for 20 min. After that, tubes were centrifuged again for 10 min at 1700 rpm. At this stage, 5 mL cold fixative (3:1 methanol: acetic acid) was added to the tube and re-do stage of centrifuge to stop further cell swelling. This fixation was repeated three times. The cells were then dropped onto clean slides, and incubated for 3 days at 37°C on a slide warmer.

On the other hand, Routine culture tubes after the 72 hours incubation period were harvested by 0.2 mL colcemid (Gibco BRL) and incubated at 37°C for 1 hour. Other steps were performed in accordance with the HR culture as mentioned above.

G-banding technique was applied with the use of pancreatin - giemsa . Analysis of the karyotype was performed in at least 20 metaphase cells for each person. Karyotypes were described according to the International System of Human Cytogenetic Nomenclature (ISCN).

Molecular analysis

From blood samples of tube with EDTA, mononuclear cells (MNCs) were isolated using Fiqoll-Paque and at last, cell deposited could be stored in TRIzol Reagent in -80 °C.

RNA isolation and cDNA synthesis

RNA and miRNA were extracted separately by using Hybriyd-R miRNA kit (Gene All Company) and then cDNA synthesis was performed using miScript II RT Kit (Qiagen Company) (oligo (dT) primers) according to the manufacturer's protocols.

Quantitative real-time PCR (qRT-PCR)

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and mir-192 were used as an internal control for NRAS genes and miR let-7a, respectively. Primers of NRAS and GAPDH genes were designed using the online primer 3 software. The sequences and Tm values of all the primers, as well as the lengths of the PCR products, can be seen in Table 1. To investigate the microRNA expression Qiagen company primers were used.

NRAS gene and miRNA let-7a levels were detected by quantitative real-time PCR reactions were performed using Step one (ABI company) and Master Mix SYBR Green high rox (Ampliqon A/S Denmark) in triplicate for each reaction. Real-time PCR reaction was performed as follows: a master mixture containing 2 µl of cDNA for each 10 µl of RealQ Master Mix Green, and 2 µl of primers (10 µmol/L), for NRAS gene 1 µl of each of the Forward and Reverse primers, was prepared on ice. The final volume was then adjusted to 20µl with deionized water. After the reaction mixture was loaded into a MicroAmp Fast Optical Reaction Plate, PCR was carried out under the following cycling conditions: initial denaturation at 95°C for 10 min, followed by 45 cycles (for NRAS 40 cycles) of denaturation at 95°C for 15 s, annealing at 55°C (for NRAS 58°C) for 30 s and extension at 72°C for 30 s. To test the specificity of the PCR, the reaction products were subjected to melting curve analysis with the LightCycler system and to

conventional agarose gel electrophoresis to rule out the synthesis of unspecific products. To confirm the sequencing of amplified fragment, the products of real time PCR were cloned and sequenced using E.coli bacteria and pBluescript SK (+) vector.

Statistical Analysis

The relative expression level and Fold Change of each miRNA let-7a and NRAS gene were calculated in all patients compared to control group using the following equations:

ΔC_T = C_T (Target Gene/miRNA) – C_T (Reference Gene/miRNA)

$\Delta\Delta C_T = \Delta C_T$ sample – ΔC_T control

$2^{-\Delta\Delta CT} = 2^{-(\Delta CT \text{ sample} - \Delta CT \text{ control})}$

Independent T test was conducted using SPSS statistical software to compare the overall situation of Fold Change.

RESULTS

Patients' characteristics

The cohort study in the MDS patients which classified based on 2008 WHO MDS classification was done, and characteristic of the patients was as follows: 11 patients with refractory cytopenia with multilineage dysplasia (RCMD), 5 patients with Refractory cytopenias with unilineage dysplasia (RCUD), 2 patients with refractory anemia with excess blasts (RAEB-1), 2 patients with refractory anemia with excess blasts (RAEB-2) and 1 cases with refractory anemia with ring sideroblasts (RARS). Risk assessment was conducted by the IPSS-R. Ten patients, with IPSS scores 1.5 – 3, were defined as the low-risk MDS group and the rest were as follow: 4 cases in very low risk (IPSS scores ≤1.5), 5 cases in intermediate risk (IPSS scores 3 – 4.5) and 2 cases in high risk group (IPSS scores 4.5 – 6) (Table 1).

Cytogenetic results

Out of 21 patients which studied by karyotype analysis, only one patient with RCMD in intermediate risk group had an increase in constitutive heterochromatin region below the centromere of chromosome 1(46,XX(1qh+). Twenty other surveyed cases with routine and High resolution (HR) culture were normal (Figure1).

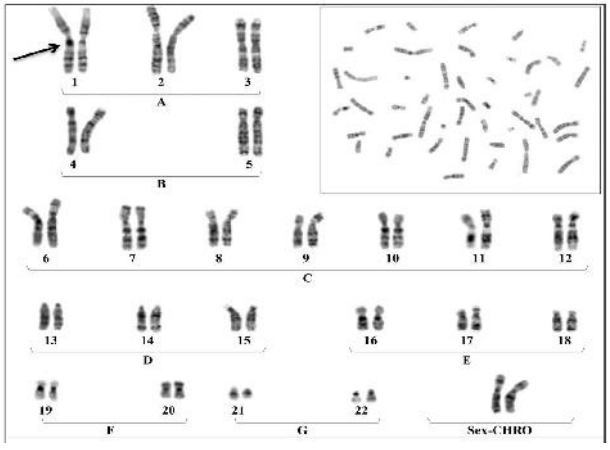

Figure 1 Karyotype of patients with an increase in constitutive heterochromatin region below the centromere of chromosome 1 (46,XX(1qh+).

Real Time PCR

The expression level of miR let-7a was 0.1165 ± 0.02725-fold change (Mean ± SEM) in patients compared to the control group. A significant decrease was observed in expression of let-7a miR in patients compared to the control group (P<0.0001) (Figure 2).

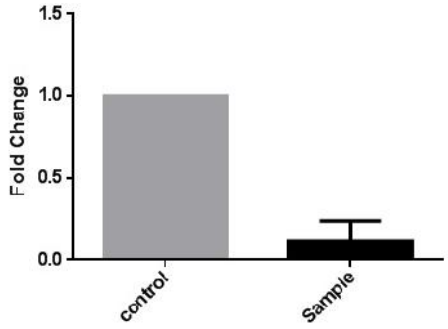

Figure 2 Comparison of let-7a expression in MDS patients and control group. P< 0.0001.

Comparison of let-7a miRNA expression in samples of women and men in the patients were 0.04765 ± 0.02767 and 0.2133 ± 0.05338- fold change (Mean ± SEM) compared to the control group and both of them showed a significant decrease in expression of let-7a miRNA (P<0.0001) (Figure 3,4).

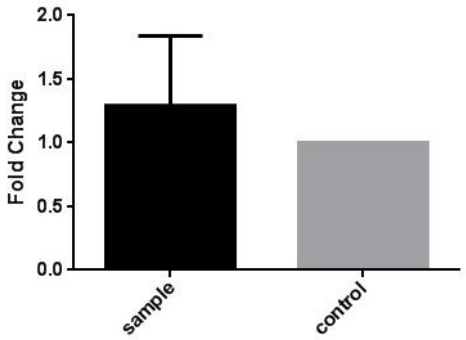

Figure 3 Comparison of NRAS expression in MDS patients and control group P= 0.0028.

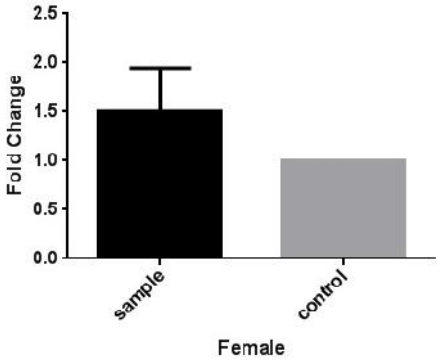

Figure 4 Comparison of NRAS in female of MDS and control group P<0.0001.

T-independent test results for the investigation of the expression obtained from the equation

$2^{-\Delta\Delta CT}$ for NRAS gene in the patients show 1.29 ± 0.1201- fold change (Mean ± SEM) that was significantly higher in patient samples than control group (P= 0.0028) (Figure 3).

Expression of NRAS gene in women with MDS were 1.506 ± 0.1661 - fold change (Mean ± SEM) and compared to control

group showed a significant increase in expression of NRAS (P<0.0001) but in men with MDS compared to men in control did not reveal any significant differences in expression of NRAS (P= 0.3602) (Figure 4,5).

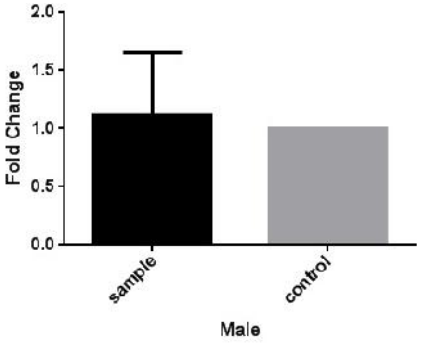

Figure 5 Comparison of NRAS in male of MDS and control group P= 0.3602

DISCUSSION

Myelodysplastic syndrome was described by Leube in 1900 for the first time. Our understanding of pathogenesis of MDS because of heterogeneous disease is not complete yet. There is not a single mechanism that can be considered as a cause of complex phenotypes for MDS patients, different methods is necessary for an accurate and exact diagnosis. Some of these methods are cytogenetic and molecular techniques such as karyotyping, FISH, SNP array and mutation analysis in candidate genes (Nimer, 2008; Malcovati *et al*., 2013; Rainer and Ulrich, 2014).

Clinical Division: In this study 2 patients had IPSS score 5, they were in high risk group and also both of them were diagnosed with RAEB-2. In both of them the disease was progressed to acute myeloid leukemia (AML) after 8 and 9 months since onset of initial symptoms. According to the research, MDS in patients with low and very low risk doesn't progress to AML, but patients in high and very high risk groups are diagnosis in RAEB subtype, and transform to AML in the mean time about 0.8 year (Greenberg *et al*., 2012; Voso *et al*., 2013). These results are consistent with our study and revealed the importance of using IPSS-R in diagnosis and treatment decision of disease. Moreover in another study on 5 childrens with RAEB-2, AML transformation occurred in the period 2 months (Rama *et al*., 2014).

The effects of changes heterochromatic: In the present study, karyotype analysis of a patient showed 46,XX(1qh+). According to the previous studies, an increase or decrease in constitutive heterochromatin is typically not associated with a particular phenotype, but some studied have shown that there is a relationship between heterochromatin polymorphism and cancer that it is possible they cause an increased rate of chromosome breakage and facilitate the cytogenetic changes in neoplastic transformation. Also heterochromatin plays a role in protecting the genome against mutagens (de Vinuesa *et al*., 1985; Movafagh *et al*., 2011).

A few studies have been done about the relationship between hematological disorders and heterochromatin variants. The constitutive heterochromatic regions just below the centromeres of chromosomes 1, 9, and 16 and the telomeric end of the long arm of the Y-chromosome can vary widely in size among individuals and in some cases can appear abnormal (Wan, 2014). In an older study on patients with MDS, 85 % of them had increase heterochromatin region in chromosome 1 (1qh+) compared with control group (de Vinuesa *et al*., 1985). In another investigation increase of heterochromatin polymorphism regions of chromosomes 1, 9 and 16 related to leukemia patients compared to healthy normal controls was observed (Movafagh *et al*., 2011). As noted above, one of the patient 's karyotype reported as 46,XX(1qh+) and According to previous studies, the cases with variant heterochromatin of chromosomes 1 susceptible to malignancies such as MDS.

Cytogenetic abnormalities are detected in approximately 50% of patients with primary MDS (Rainer and Ulrich, 2014). However, in our study, only one of 21 patients (approximately 5%) by routine Cytogenetic analysis was reported as 46, XX (1qh +). The justification for this difference is as follow: At first, the difference is in the utilization of methods, in most studies FISH and M-FISH methods in addition to the normal karyotype were used. These techniques have the ability to detect chromosomal abnormalities with high resolution and increase the possibility identification of abnormal karyotypes in patients (Zhang *et al*., 1999; Blau *et al*., 2007; Schlegelberger *et al*., 2012). Another reason is the examined tissue, in those studies often Bone Marrow Aspiration were used for cytogenetic analysis (Flores-Figueroa *et al*., 2005; Blau *et al*., 2007; Pozdnyakova *et al*., 2008); while in our study peripheral blood was used for diagnosis.

In laboratories utilization of FISH technique for MDS cytogenetic abnormalities is growing. Most reports provided the results of a bone marrow sample, while analysis of peripheral blood samples with FISH has a potential for non-invasive screening in MDS cytogenetic abnormalities before performing bone marrow cytogenetic tests. Research about comparing between diagnostic utility of FISH in peripheral blood and bone marrow was done rarely. Coleman JF and *et al.* demonstrated the importance and advantages of FISH analysis in comparison with routin karyotype in 433 patients with MDS and AML. Another aspect of this study is comparison the results of the peripheral blood and bone marrow, in 48 patients peripheral blood FISH analysis was done and abnormality in 69% of patients with abnormal bone marrow were seen. These results suggested that if FISH technique is used, in more than half of the patients only with examining peripheral blood samples can be found their abnormalities; therefore need to a very aggressive technique- bone marrow aspiration- be minimized (Coleman *et al*., 2011).

In this study, our aim was potential utilization of peripheral blood instead of bone marrow aspiration in primary detection. As noted above cytogenetic abnormalities in the best conditions and use from more accurate techniques only have been observed in 50% of patients with MDS and others have normal karyotype (Gupta *et al*., 2010). Thus, we need more new markers for early and accurate diagnosis of the disease. If a normal karyotype is reported, communication with a genomic imbalance can be raised. Special biomarkers with diagnostic value, such as cell surface markers, gene expression profiling, miRNA expression and copy number analysis -High resolution- for MDS have been proposed (Bejar, 2014).

MicroRNAs (miRNAs) are small (18-25 nucleotides) non-coding RNAs involve in many cell critical processes and up or down regulation of them results for disease or its progression (Merkerova *et al*., 2011). In fact, change in miRNAs expression level play key role in tumorigenesis through deregulating of target genes expression. Disruption in the expression of miRNAs that are necessary for normal hematopoiesis, are considered a key pathogenic factor in hematologic malignancies such as MDS.

The existence and stability of miRNAs in various body fluids leads to the attractiveness of this class of molecules as non-invasive biomarkers in a wide range of malignant diseases (Tanaka *et al*., 2009). On the other hand, miRNA let-7a has an important role in the development of leukemia and MDS by interfering in cell cycle regulating and apoptosis. Several studies have been carried out on miRNA let-7a in different cancers, in most of them decreased expression of this miRNA has been reported. Also, many researches have been done on that target gene RAS which includes both mutations and expression of this gene. Mutations in KRAS and NRAS genes were seen frequently in myeloid disorders, such as AML and CML (Downward, 2003).

Three human RAS gene have binding sites for let-7 in their 3 'UTR and cell cycle is regulated by it. This evidence indicates the involvement of miRNA Let-7 and its eventual target NRAS in physiopathology of MDS. Also on lung tumor tissue, significantly reduced expression of the let-7a miRNA and a considerable increase of the RAS protein levels reported in these patients that suggests let-7a acts as a regulator of RAS in lung cancer (Johnson *et al*., 2005). The results of a survey showed that the level of let-7a expression has been decreased in vitro and in vivo experiments and it was observed that overexpression of this miRNA in lung adenocarcinoma cell lines A549 prevents cell growth (Takamizawa *et al*., 2004). The findings of these studies with our results regardless of the tissues examined are conformed in terms of reduced let-7a and NRAS.

In a study circulating microRNAs let-7a and miR-16 in 50 patients with MDS and 76 healthy controls were examined by using Real Time PCR techniques. Both microRNA expression levels in patients compared to control was significantly decreased (Zuo *et al*., 2011).

Another study performed to analyze the expression of microRNA let-7a and its target protein RAS in $CD34^{+}$ bone marrow cells in patients with MDS. The results of this study also showed expression of let-7a compared with the control group has significant decrease. In addition, they found that its target protein levels RAS correlated inversely with the level of this miRNA. RAS mutated protein is activated permanently that have a bad prognosis and also increases the probability of transformation to AML (Vasilatou *et al*., 2013a).

The results of our study showed that the expression of miRNA let-7a and its target gene NRAS in patients with MDS respectively was decreased and increased significantly. This inverse relationship between miRNA expression and its target is observed in most of articles and other cancers that are conformed to our results.

In the following, a study of 25 miRNA, including miR-16, miR-15a, miR-181a, miR-222 from bone marrow and peripheral blood samples of 25 patients with primary MDS and 12 controls were performed. Twelve miRNA expression in bone marrow and 6 miRNA expression in peripheral blood showed different expression between patients and control group. In this study twenty-two cases were in Low-risk group and 3 cases were in High-risk group. In this study the correlation between miRNA and IPSS classification on two miRNAs was analyzed, miR-15a showed increased expression in the bone marrow of patients HR IPSS group, miR-16 expression in peripheral blood of patients LR IPSS was also increased (Pons *et al*., 2009).

In another study, miRNA expression of 44 patients with MDS and 17 controls were evaluated using microarray. Expression of thirteen miRNAs showed statistically significant difference between patient and control group. Also expression of 10 miRNA had closely related to risk group defined in the IPSS, HR and LR that was statistically significant (Sokol *et al*., 2011).

CONCLUSION

In this study we found that most patients with sporadic MDS have normal karyotype and heterochromatin polymorphism can sometimes appear abnormal. Also let-7a and its target NRAS levels between healthy control and MDS patients were significantly different, therefore; it can be as a noninvasive biomarker for diagnosis or prognosis of MDS patients, and it needs to confirm with future studies.

Further Cytogenetic investigation and the identification of suitable markers with better prognostic value (such as miRNA expression level in peripheral blood) for MDS are promising. Continuing this project in the future, help not only early diagnosis of patients during the latency period and before transformation into AML, but also using the molecular panel on the base of miRNA and its target gene expression, such as cytogenetic panel (IPSS), which now is used by researchers.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgement

We would like to express our appreciations to the vice chancellor for research of Mashhad University of Medical Sciences for providing the grant of this project.

References

Bejar R (2014). Clinical and genetic predictors of prognosis in myelodysplastic syndromes. *haematologica*, 99, 956-64.

Blau O, Hofmann W-K, Baldus CD, *et al* (2007). Chromosomal aberrations in bone marrow mesenchymal stroma cells from patients with myelodysplastic syndrome and acute myeloblastic leukemia. *Exp Hematol*, 35, 221-9.

Coleman JF, Theil KS, Tubbs RR, *et al* (2011). Diagnostic yield of bone marrow and peripheral blood FISH panel testing in clinically suspected myelodysplastic syndromes and/or acute myeloid leukemia a prospective

analysis of 433 cases. *American journal of clinical pathology,* 135, 915-20.

de Vinuesa ML, Larripa I, de Pargament MM, *et al* (1985). Heterochromatic variants and their association with neoplasias. II. preleukemic states. *Cancer Genetics and Cytogenetics,* 14, 31-5.

Downward J (2003). Targeting RAS signalling pathways in cancer therapy. *Nature Reviews Cancer,* 3, 11-22.

Erdogan B, Facey C, Qualtieri J, *et al* (2011). Diagnostic microRNAs in myelodysplastic syndrome. *Exp Hematol,* 39, 915-26 e2.

Flores-Figueroa E, Arana-Trejo RM, Gutiérrez-Espíndola G, *et al* (2005). Mesenchymal stem cells in myelodysplastic syndromes: phenotypic and cytogenetic characterization. *Leukemia research,* 29, 215-24.

Germing U, Aul C, Niemeyer CM, *et al* (2008). Epidemiology, classification and prognosis of adults and children with myelodysplastic syndromes. *Annals of hematology,* 87, 691-9.

Greenberg PL, Tuechler H, Schanz J, *et al* (2012). Revised international prognostic scoring system for myelodysplastic syndromes. *Blood,* 120, 2454-65.

Gupta G, Singh R, Kotasthane DS, *et al* (2010). Myelodysplastic syndromes/neoplasms: recent classification system based on World Health Organization Classification of Tumors–international Agency for Research on Cancer for Hematopoietic and Lymphoid Tissues. *Journal of blood medicine,* 1, 171.

Haase D, Germing U, Schanz J, *et al* (2007). New insights into the prognostic impact of the karyotype in MDS and correlation with subtypes: evidence from a core dataset of 2124 patients. *Blood,* 110, 4385-95.

Heydari S, Ghods E, Mojarrad M, *et al* (2016). Expression Analysis of Let-7a miRNA and its Target Gene NRAS in Cytogenetically Normal Family with Myelodysplastic Syndrome. *International Biological and Biomedical Journal,* 2, 31-8.

Johnson SM, Grosshans H, Shingara J, *et al* (2005). RAS is regulated by the let-7 microRNA family. *cell,* 120, 635-47.

Malcovati L, Hellström-Lindberg E, Bowen D, *et al* (2013). Diagnosis and treatment of primary myelodysplastic syndromes in adults: recommendations from the European LeukemiaNet. *Blood,* 122, 2943-64.

Merkerova MD, Krejcik Z, Votavova H, *et al* (2011). Distinctive microRNA expression profiles in CD34+ bone marrow cells from patients with myelodysplastic syndrome. *European Journal of Human Genetics,* 19, 313-9.

Movafagh A, Mortazavi-Tabatabaei S, Kolahi A (2011). The role of α-satellite DNA and heterochromatin polymorphism in leukemia patients and illicit drug addicts. *Genetics and Molecular Research,* 10, 3999-4005.

Mufti GJ, Bennett JM, Goasguen J, *et al* (2008). Diagnosis and classification of myelodysplastic syndrome: International Working Group on Morphology of myelodysplastic syndrome (IWGM-MDS) consensus proposals for the definition and enumeration of myeloblasts and ring sideroblasts. *Haematologica,* 93, 1712-7.

Nickels EM, Soodalter J, Churpek JE, *et al* (2013). Recognizing familial myeloid leukemia in adults. *Ther Adv Hematol,* 4, 254-69.

Nimer SD (2008). Myelodysplastic syndromes. *Blood,* 111, 4841-51.

Owen C, Barnett M, Fitzgibbon J (2008). Familial myelodysplasia and acute myeloid leukaemia--a review. *Br J Haematol,* 140, 123-32.

Pons A, Nomdedeu B, Navarro A, *et al* (2009). Hematopoiesis-related microRNA expression in myelodysplastic syndromes. *Leuk Lymphoma,* 50, 1854-9.

Pozdnyakova O, Miron PM, Tang G, *et al* (2008). Cytogenetic abnormalities in a series of 1029 patients with primary myelodysplastic syndromes. *Cancer,* 113, 3331-40.

Rainer H, Ulrich G 2014. *Novel Insights into Pathophysiology, Diagnostics and Treatment of Myelodysplastic Syndromes*, Future Medicine Ltd.

Rama H, Gupta D, Chatterjee T, *et al* (2014). Fanconi Anemia with MDS RAEB-2 Rapidly Progressing to AML in a 5-Year-Old Boy. *Indian Journal of Hematology and Blood Transfusion,* 30, 379-82.

Rhyasen GW, Starczynowski DT (2012). Deregulation of microRNAs in myelodysplastic syndrome. *Leukemia,* 26, 13-22.

Schlegelberger B, Göhring G, Thol F, *et al* (2012). Update on cytogenetic and molecular changes in myelodysplastic syndromes. *Leuk Lymphoma,* 53, 525-36.

Sokol L, Caceres G, Volinia S, *et al* (2011). Identification of a risk dependent microRNA expression signature in myelodysplastic syndromes. *Br J Haematol,* 153, 24-32.

Takamizawa J, Konishi H, Yanagisawa K, *et al* (2004). Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. *Cancer research,* 64, 3753-6.

Tanaka M, Oikawa K, Takanashi M, *et al* (2009). Down-regulation of miR-92 in human plasma is a novel marker for acute leukemia patients. *PLoS One,* 4, e5532.

Vasilatou D, Papageorgiou SG, Kontsioti F, *et al* (2013a). Expression analysis of mir-17-5p, mir-20a and let-7a microRNAs and their target proteins in CD34+ bone marrow cells of patients with myelodysplastic syndromes. *Leukemia research,* 37, 251-8.

Vasilatou D, Papageorgiou SG, Kontsioti F, *et al* (2013b). Expression analysis of mir-17-5p, mir-20a and let-7a microRNAs and their target proteins in CD34+ bone marrow cells of patients with myelodysplastic syndromes. *Leuk Res,* 37, 251-8.

Voso MT, Fenu S, Latagliata R, *et al* (2013). Revised International Prognostic Scoring System (IPSS) predicts survival and leukemic evolution of myelodysplastic syndromes significantly better than IPSS and WHO prognostic scoring system: validation by the Gruppo Romano Mielodisplasie Italian Regional Database. *Journal of Clinical Oncology,* 31, 2671-7.

Wan TS (2014). Cancer Cytogenetics: Methodology Revisited. *Annals of laboratory medicine*, 34, 413-25.

Zhang W, Knieling G, Vohwinkel G, *et al* (1999). Origin of stroma cells in long-term bone marrow cultures from patients with acute myeloid leukemia. *Annals of hematology*, 78, 305-14.

Zuo Z, Calin GA, de Paula HM, *et al* (2011). Circulating microRNAs let-7a and miR-16 predict progression-free survival and overall survival in patients with myelodysplastic syndrome. *Blood*, 118, 413-5.

How to cite this article:

Elahe Ghods *et al*.2017, Assessment of Cytogenetic Changes And Expression of Let-7a Microrna in Relation to its Target Gene Nras In Myelodysplastic Syndrome (MDS). *Int J Recent Sci Res.* 8(9), pp. 19759-19765.
DOI: http://dx.doi.org/10.24327/ijrsr.2017.0809.0754

****