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# The examination of miR199b and Hypoxia Inducible Factor 1α (HIF-1α) expression in patients with Acute Myeloid Leukemia cancer (AML) by real time PCR methods

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**ABSTRACT:**

Blood cancer is related to the blood-forming tissues of the body and Acute Myeloid Leukemia cancer (AML) is one of its fatal and common types. Blood cancer or leukemia covers about 8% of all cancers and is the fifth common cancer of the world. Micro-RNAs (miRNA) are a small group of non-coding small RNA suppressing the expression after the target genes transcription by the imperfect pairing of the bases with the un-translated region '3 ('3-UTR). HIF-1 transcription factor (Hypoxia Inducible Factor-1) plays an important role in physiology and pathology. Overexpression of HIF-1 has been viewed in many types of cancer including blood cancer. In tumor cells, the expression of the genes affecting angiogenesis, cell cycle, and its metabolism is controlled by an inducible factor with Hypoxia-1 (HIF-1). Recently, description of the molecular characteristics of angiogenic pathways introduces this factor as a key factor of the transcription of these molecules' regulation. In this study, the expression of miR199b and HIF-1 in AML is examined in 30 patients and 10 healthy persons as a witness. It was observed that expression level of miR199b in AML reduced, while HIF-1 overexpressed.

**Keywords:**
MiR199b, HIF-1, Leukemia, Acute Myeloid Leukemia (AML).

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## INTRODUCTION

Blood cancer is the disorder in production and performance of the blood cells. This type of cancer usually starts from bone marrow and causes the formation of a large number of abnormal white blood cells. These white blood cells are not fully formed which are called blast, leukemia or blood cancer cells. Bone marrow is a spongy tissue in the middle of bone which is responsible for producing blood globules. The blood consists of three parts *viz*., white globules, red globules, and platelets. In blood cancer the blood cell production goes abnormal and excessive production of blood cells disrupt the normal blood cells' works such as fighting with infections or preventing bleeding (Jemal *et al*., 2008; Salehi *et al*., 2013).

The blood cancer covers almost 8% of whole cancers of human population and 7% of deaths due to the cancers, and it has been allocated the fifth rank of fatality in the world and the second rank in Iran. In the meantime, AML (Acute Myeloid Leukemia) is the second most common blood cancer (5.18%) and third in Iran as a deadly blood cancer (Salehi *et al*., 2013; Zand *et al*., 2010). The patients with AML often have nonspecific symptoms which have begun suddenly or gradually and usually have the symptoms such as hypercalcemia (high calcium in the blood), anemia (lack of red blood cells or decrease in their performance), kidney failure, vulnerability to infection, osteoporosis, bone pain, swelling or broken bones, excess protein in the blood or urine and weight loss or anorexia. About half of the patients have these symptoms for at least three months prior to the diagnosis of leukemia.

Half of the patients cited fatigue as a primary symptom, while others complain of stiffness or weakness in diagnosis. Infection and fever were observed in 10% of patients as the primary symptoms. A tumor represents a leukemic cell accumulation named granulocytic sarcomas or chloroma. Severe gastrointestinal bleeding, pulmonary hemorrhage or intracerebral hemorrhages are seen in acute promyelocytic leukemia.

The hemorrhages due to the blood coagulation disorders can be observed in AML of monocyte. Retinal hemorrhages can be seen in 15% of patients (Melnick, 2002). Laboratory studies showed that the blast cells in the bone marrow and peripheral blood of patients develop leukemia. The production of mature cells in normal follicles also disrupts (Andersson *et al*., 2015). The prevalence of AML is approximately 3.2 % in 100,000 which increase with the disease's outbreak so that its incidence in people under age 65 is 1.3% and in people over 65 years is 12.2%.

During 2002, about 30,800 new cases of leukemia were diagnosed in America; almost half of these cases were acute leukemia. The most common adult leukemia is acute myelogenous leukemia which had included 10,600 cases. About 8,800 deaths as a result of acute leukemia occurred in 2002 in America. The average age of getting Acute Myelogenous Leukemia (AML) is 65. The Acute Lymphocytic Leukemia (ALL) is more common among children. The age of pediatric patients is less than 10 years (Greer *et al*., 2014).

### AML and its outbreak

AML in adults is a cancer related to the blood and bone marrow. This type of cancer, if left untreated, can have a deterioration rapidly. It is the most common type of acute leukemia among adults. Other names of the AML blood cancer are acute myelogenous leukemia, acute myeloblastic leukemia, and acute non-lymphocytic leukemia. Naturally, bone marrow produces the blood stem cells (immature cells) which gradually become mature blood cells. The lymphoid stem cell also gradually turns into a white blood cell.

Bone marrow stem cells are normally transformed into a form of white blood cell called myeloblast for AML blood cancer. Myeloblasts are rare in AML and do not become white blood cells that are

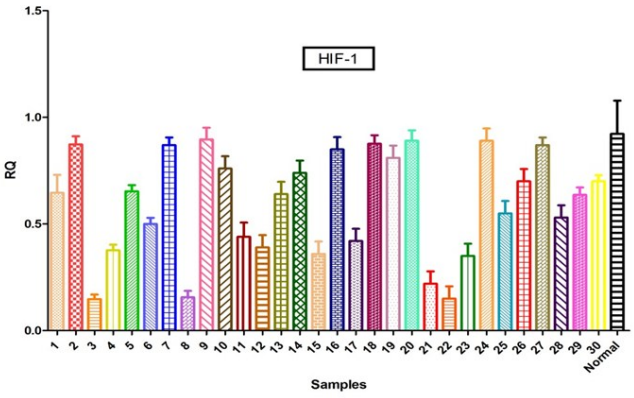


**Figure 1. Expression level of gene HIF-1 in patients in comparison to the normal people**

stable. Stem cells often transform to white blood cells that are stable. Stem cells often transform to white blood cells or platelets in AML blood cancer much more than the normal range; such white blood cells or platelets are called leukaemia or blast.

In the bone marrow and blood, leukaemia cells accumulate and therefore less space will exist for the healthy white, red blood cells and platelets. In this example, inflammation, anaemia, or simple bleeding will occur. In addition to regions of the blood and other area of the body, leukaemia cells often invade the central nervous system (brain and spinal cord), skin and gums. Most subtypes of AML are calculated based on the quantity of mature (growing) cancer cells and how distinct they are from normal cells at the time of diagnosis (Greer *et al*., 2014). The prevalence of AML is approximately 2.3 per 100,000 that increases with the increase of age; somehow, its prevalence in people under 65 years old is 1.3% and in people higher than 65 is 12.2%.

**Hypoxia-Inducible Factor (HIF-1)**

Cancer is defined as an uncontrollable growth of abnormal cells. According to the recent studies, when the size of tumor enlarges to 1 mm, it encounters with the lack of oxygen and food (Hypoxia). Then tumor cells provide their metabolic needs and also toxic materials from the environment by implementing the surrounding endothelial cells and angiogenesis (Guitart, 2013). Angiogenesis is the physiological process through which new blood vessels form from pre-existing vessels. It is the most important mechanism in order to provide the necessities of cells, which are far from blood vessels (Folkman, 1984). Although angiogenesis is necessary for many psychological operations like development, creation, wound healing and sexual reproduction, it has a significant role in the pathologic conditions like tumor growth, metastatic and a lot of chronic diseases.

The variety of parameters like HIF-1 has an important role in the process of forming pathologic conditions like cancer. One of these factors is a heterodimer protein which is activated in hypoxia conditions and has an important impact on those genes which create an adaptation of cell to hypoxia conditions. The ability to keep oxygen hemostasis is necessary for both vertebrates and invertebrates types. A lot of psychological systems were developed for being assured of optimal oxygenation in multi-cell types.

According to a vertebrate in comparison with invertebrates, there is a direct relationship between the size of body and oxygenating process which is related to heart, lungs, blood vessels and red blood cells (Hochachka, 1998). Adjustment of angiogenesis by using hypoxia is one of the most important hemostasis mechanisms which relates metabolic needs and supplement of oxygen by using vessels (Carmeliet, 2003; Giaccia *et al*., 2004). In tumors, there is a limited access of oxygen and foods, because of the high speed of cells reproduction. Also, the development of metabolites were under control because of tension in tissues (Stohrer *et al.,* 2000).

In response to tumor's hypoxia, the angiogenesis stimulating factors try to form a new blood source by using existence blood vessels. The above process is necessary for the reproduction of cells in appropriate conditions (Semenza, 2000; Richard *et al*., 1999). The capillary damage of wounds creates a

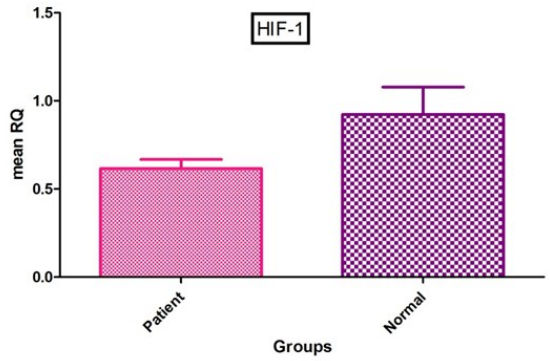


**Figure 2. Comparison of gene HIF-1 expression**

hypoxic environment, changing the amount of oxygenation in the place of the wound and will change the repair angiogenesis responses, respectively (Richard *et al*., 1999). So, hypoxia is defined as an important factor in the process of angiogenesis in both psychological and pathologic conditions. According to the recent studies, findings indicate the role of HIF-1 in order to adjust the tumor cells in response to the hypoxia conditions.

This process includes adjustment of involved genes in angiogenesis, so it leads to the development of metastasis and its surrounding tissues. HIF-1 is a kind of heterodimer protein which has two subunits as the following: the first one is an inducible subunit which is expressed with HIF-1α and the second one is HIF-1β, which is permanently expressed in the cells (Wang and Semenza, 1995). At first, HIF-1β was identified as a transmitter of hydrocarbon aril. Then, it was clear that it is a kind of subunit which is connected to the hydrocarbon aril receiver. Meanwhile, HIF-1α is a kind of new protein and has an important role in gene expressions which are related to the compatibility with hypoxia conditions (Reyes *et al*., 1992).

Hypoxia and HIF signaling pathway are associated with embryogenesis and pathology of many human diseases. In the growth of tumors, the role of these two factors in increasing oxygen delivery to cells *via* angiogenesis and activation of glycolysis is proven. Therefore, regarding the importance of HIF-1 in activation of necessary genes for pushing forward this process, the necessity of HIF-1α and HIF-2α for cancer is not unexpected (Forsythe *et al*., 1996). According to what mentioned above, HIF plays an important role in regulating the angiogenesis in physiologic and pathologic conditions.

Although, the existence of the angiogenesis and high expression level of HIF are necessary for physiologic conditions such as embryo development or pathologic conditions, with respect to the role of HIF in angiogenesis and metabolic and metastasis adaptation of the cancer cells, this factor has changed into one of considered and important factors for targeting in order to reduce or stop its expression in cancer treatment. However, achieving this goal requires more researches and studies to more complete and precise understanding of molecular mechanisms involved in the pathway of HIF in response to the hypoxia (Kirito *et al.*, 2005; Ceradini *et al*., 2004; Simon *et al*., 2008; Weiwei *et al*., 2013).

**HIF in blood cancer**

Increase of the HIF expression level is a weak marker in solid cancers. Generally, the increase of HIF expression level is associated with the tumor development and resistance to treatment leading to disease recurrence. This issue is more complex and more controversial. Overexpression of HIF-1α in leukemia is proposed as a strong marker. The disease severity and survival are influenced by the levels of HIF -1α. Hence, the type of blood cancer explains the stage of disease or molecular abnormalities, and the variety of different levels of oxygen. Several studies have indicated that the inhibition of HIF-1α by targeting the iRNA which are the small interferer RNAs decrease the tumor development and the blood cancer development (Deeb *et al*., 2011).

On the other hand, HIF-1α and HIF-2α affect the signaling pathways related to the protection and development of blood cancer. And on the other hand,

HIF-1α operates like p15, p16, p19, and p53 as an inhibitor of tumor suppressor genes expression. Overall, these data provide new therapeutic information representing this fact that targeting the HIF in leukemia does not have any impact on the normal hematopoietic cells and while the patients with leukemia may be treated (Percio *et al*., 2014).

**MicroRNA molecules**

MicroRNAs (miRNA or miRs) are a new class of small molecules of the endogenous non-coding RNA which regulate the gene expression at the post-transcriptional level through mRNA decomposition or translation inhibition. Since the discovery of lin-4 and let-7 small non-coding RNAs (known as miRNA) in *Caenorhabditis elegans*, hundreds of miRNA sequences are known in a wide range of organisms, from plants to humans. For human genome, more than 1,000 miRNA encoding genes are estimated that include about one percent of genome.

It seems that these miRNAs are responsible for the regulation of the expression of one-third of human genome. Moreover, each miRNA directly or indirectly targets about 200 transcripts, However, a protein encoding gene can also be targeted or regulated by more than one miRNA (Rodriguez *et al*., 2004). The key role of miRNAs has been proven as the regulators of various cellular processes such as developmental timing, tissue differentiation, cellular proliferation, organ development, maintenance of stem cell potency, and apoptosis. Aberrant expression of miRNAs is reported in many human cancers and there are strong evidences for key role of miRNAs as the oncogene or tumor suppressor in development of many human malignancies (Zhu *et al*., 2007).

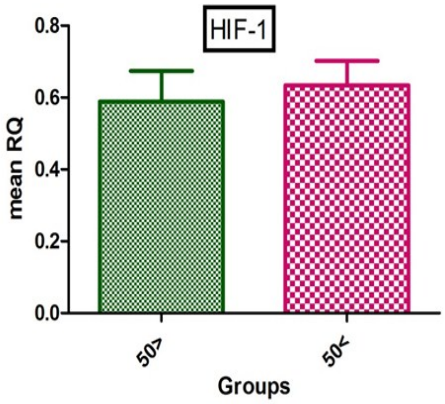


**Figure 3. Comparison of age groups in HIF-1 expression**

The analysis of the expression profiles of miRNA in tumor samples have shown different expression profiles of adult and/or progenitor miRNAs compared to normal cells of the same tissue. Similarly, corroborative evidences of the role of miRNA in stem cell biology are studied by various authors. In this field, 36 unique miRNAs are identified by cDNA cloning that is expressed especially in human embryonic stem cells compared to differentiated embryoid bodies (Sioud and Røsok, 2004).

**MicroRNA 199b in blood cancer**

Dysregulation of miRNA which can act as oncogenes or tumor suppressor would be tumorigenic. Human miR-199b is on chromosome 9 and is located in 2.2 Kb intron zone between the exons 14 and 15. MiR-199b plays an important regulatory role in the differentiation (Beveridge *et al*., 2008). Recent studies have shown that about 50% of human miRNA have been associated with cancer. Therefore, miRNAs have been studied increasingly as a biomarker and therapeutic target. The expression of miRNA decreases in different human tumors like the cancer of colon, liver, brain, bladder, breast, uterus, thyroid, and blood cancer. HIF-1α expression results in resistance to radiotherapy and chemotherapy. Although miR199b overexpression inhibit the cell growth and tumor formation, HIF-1α gene suppression by siRNA inhibits cell growth, cell division or migration, and promotes apoptosis in a variety of tumors (Voorhoeve *et al*., 2006).

MiR-199b significantly decreases in cancer and HIF-1α is its directly applied target gene. HIF-1α

mRNA is identified as the direct target gene of the miR199b. Moreover, miR199b over expression in cancer cells may significantly disrupt the expression of HIF-1α under hypoxic and normoxic conditions and associated with a reduction in cell growth and increased apoptosis. Hence, miR-199b has a potential application as a biomarker or therapeutic agent for cancer. These findings contribute in more understanding of miRNA performance in cancer and indicate that miR-199b may be used as a treatment for cancer.

## MATERIALS AND METHODS

At first, 5 mL of peripheral blood samples from 30 AML patients admitted to Dena medical laboratory in Tehran, and of 10 healthy blood samples lacking this disease as a control sample of Shariati hospital between the years 94 to 95 were collected in a test tube. The needed items were ethanol 75%, RNX-PLUS solution, chloroform, isopropanol, DEPC water (Nuclease-free water), random hexamer, oligo- dT (primer), dNTPs, enzyme M- MuLV, M- MuLV 10X buffer. In this method, at first RNA was extracted by following steps:

First, 100 µL of blood was shed in a sterile micro-tube and then, 500 µL of RNX- solution was added to the above solution and was vortexed for 5 to 10 sec. 400 µL of chloroform was added to the above micro-tube and was vortexed for 15 sec. It was centrifuged at 4°C for 25 min at 12000 rpm. Achromatic phase of zinc was moved to a new micro-tube and same volume of cold isopropanol was added and after 10 times inverting, it was placed at -20°C for two hours. It was centrifuged for 25 min at 13000 rpm at 4°C and then, the zinc solution was decanted. 1000 µL of cold 70% ethanol was added to the micro tube. Micro tube was inverted for 10 times and centrifuged at 13000 rpm at 4°C for 10 min and then the zinc solution was evacuated. 30 µL of DEPC water was added to sediment and it was dissolved. For cDNA synthesis, suitable upstream and downstream primers are used which may be applied publicly for making cDNA treasury or specifically for the target gene.

Due to the single-strands, the proliferation is linear over the early cycles of PCR, because the first DNA strand operates as a pattern just for one of the primers. Exponentially, proliferation by both primers is performed only when sufficient versions of the second strand are produced. The final content of the real-time PCR reaction is 20 µL. 10 µL of the master mix was taken and 0.25 µL of the each of primers 'r' and 'f' and 7.5 µL of water were added to it. These amounts were poured in 8 strips. In each strip, 18 µL was poured, and then 2 µL of cDNA was added. So, the final extent of each strip was 20 µL. The strips were placed in the device by their numbers and its program was set. The samples were setup at a temperature of 59°C.

**The real-time PCR reaction for the gene HIF-1**

The genes HIF-1 were relatively proliferated to measure the gene expression by real-time PCR based on the standard method. The relative quantification in real-time PCR was performed by measuring the increase of fluorescence radiation as a result of the binding of SYBR green dye (SG) using the device ABL 7500. 10 µL of the master mix was taken and 0.25 µL of the each of primers 'r' and 'f' and 7.5 µL of water were added to it. These amounts were poured in 8 eight strips. In each strip 18 µL was poured, and then 2 µL of cDNA was added. So, the final extent of each strip was 20 µL. The strips were placed in the device by their numbers and its

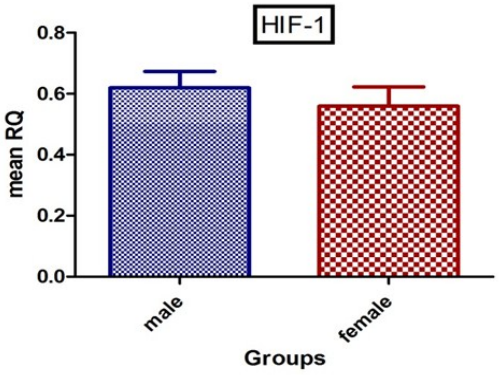


**Figure 4. The comparison of genders**

program was set. The samples were setup at a temperature of 59° C.

**Real-time PCR reaction to miR199b**

The miR199 genes were relatively proliferated to measure the gene expression by the reaction of real-time PCR based on the standard method. The quantification relative in real-time PCR was performed by measuring the increase of fluorescence radiation as a result of binding SYBR green dye using ABL 7500. 10 µL of the master mix, primers (R and F), each 5.0 µL, and 5.7 µL of water were taken. An amount of each of them was poured in 8 strips and then 1.5 µL of each sample were added to every strip. After all, the strips were placed in the device in order of the number in order to its setup be carried out at a temperature of 52° C.

The fluorescent SYBR green dye was used in these reactions. This dye connects to any double-stranded DNA and detects the DNAs and finally determines the results. After PCR completion, the obtained products melt by increasing the temperature and by reducing the temperature, they return to the initial state (double-stranded). The samples fluorescence changes during this stage would be shown on the melting curves in order to ensure that the expected results are gained and they are not due to the proliferation of non-specific products.

**The level of genes HIF-1 and miR199b expression in healthy and cancerous samples**

After extracting RNA and cDNA synthesis from the patients and control group, the expression was evaluated by real-time PCR reaction. After performing the reaction, the raw data were extracted from the device as Ct and the expression level was measured using ΔΔCt. Then, the gene expression graph was drawn using Graph pad software. In this research, the relation between the level of genes expression and the age and sex was examined, too.

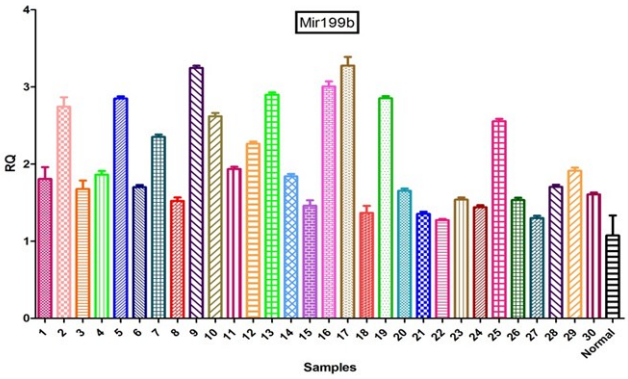


**Figure 5. The comparison of the expression of gene miR199b in the patients and normal people**

**RESULTS**

**The results of the gene HIF-1 expression**

After carrying out the proliferation reaction, the Ct of the samples were calculated by the device and turned into the RQ (Relative Quantification) or expression level, and the expression level was measured by the ΔΔCt method. The expression level of patient samples was expressed in comparison to that in fact, the results are the ratio of target gene expression to the expression of the same gene in a normal tissue. The RQ of the samples were extracted from the device and the results were drawn using Graph pad software (Figure 1).

In this study, the relative quantification is applied. 10 normal samples were used that the average expression level of these samples obtained was 0.92 by analyzer device which was determined by a black color in the right of the diagram. Here a normal sample was compared with the patient's sample. The expression level of all samples were compared with the normal sample, and as it can be seen in Figure 1, the expression level of all patient samples have increased in comparison to the normal samples and P value of 0.0001 was significant.

**The relation of data in gene HIF-1**

The analysis of the data indicated that expression level of gene HIF-1 in patient's has decreased averagely 1.5 times compared to the normal people; this analysis was declared generally and without

considering the age and sex (Figure 2) and P value was significant (P = 0.0206). The results of two groups of people over 50 years and under 50 years did not showed a significant relationship between the age of people and the level of gene expression and P value was also not significant (Figure 3). In addition, the expectation that the disease increases with age was not realized.

The results of the relation between the gender and expression level of the gene indicated that this relation in terms of statistical analyses was not significant as well as P value; it means that disease process was irrelevant to the gender (Figure 4). Similar to the expression of gene HIF-1 after the proliferation reaction, the Ct of the samples was calculated using the device and changed into RQ or expression level. The expression level of the genes was measured using ΔΔCt. The patient samples' expression level were expressed in comparison with normal samples, and actually, the results were the ratio of expression level of miR199b to the expression level of the same miR199b in a normal tissue. The samples' RQ were extracted from the device and the results were drawn using graph pad (Figure 5).

In this study, 10 normal samples were used that the average expression level of these samples was obtained as 1.07 as measured by analyzer device; it was shown on the right side of the diagram with black color and is a criterion that the patient samples are compared with. The expression level of all samples was compared with the normal samples, and as it can be found in Figure 5, the expression level of patient samples have increased in comparison to the normal samples and P value (less than 0.0001) was not significant representing significant expression level of miR199b.

**Analyzing and examining the relations of data in miR199b**

Analyzing the data showed that the value of the miR199b expression increased 2.28 times more than the normal people. This examination was expressed generally and without consideration of the age and gender (Figure 6). P value became significant (P = 0.0006) that statically showed the positive relation between the value of gene expression and cancer. Examination of two groups of individuals over 50 years and below 50 years does not show a significant relation between age and the value of the miR199b expression (P value was insignificant) (Figure 7) and the assumption of the increase in age will increase the cancer condition that have not been achieved.

The results of the comparison between the gender and the value of miR199b expression showed that this relation was insignificant in term of statistical analyses (P value was insignificant) which means that the disease has no relation with the gender (Figure 8).

## DISCUSSION

Since leukemia consists almost 8% of all cancers of human population and about 7% of death caused by malignancies and allocates the fifth place of death in the world and second place in Iran to itself. AML is the second frequent leukemia (18.5%) and third fatal leukemia in Iran. Its treatment is very important. HIF-1 is also a transcription factor that exists almost in all type of cells and targeted genes by the amount of oxygen, regulation and thousands of genes expressions. The proteins of targeted genes are involved in tumor biology, such as the transport of oxygen, iron metabolism, glycolysis, glucose transport, angiogenesis, cell proliferation, invasion, and metastasis. Therefore,

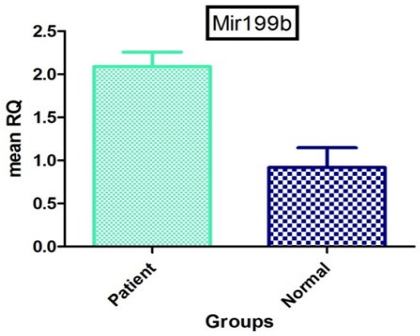


**Figure 6. Comparison of miR199b expression in patient and normal groups**

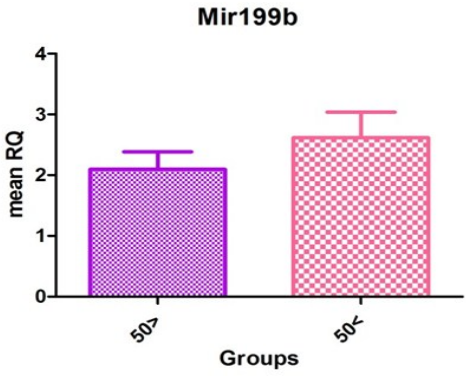


**Figure 7. The age comparison**

this gene is known as an important gene in cancers. In addition, miR-199b has an important regulatory role in differentiation.

According to the result of this study, it seems that miR199b has decreased expression in AML and increased expression in miR199b. Reduction of HIF-1 in cancer cells will decrease the growth of cell and increase the apoptosis. These results will help in the understanding of the function of miRNA in cancer and showed that this method may be used as a treatment for cancer. Results showed the important role of HIF-1 in regulating responses of tumor cells to the hypoxic conditions by regulating expression of other genes involved in angiogenesis, and finally helping the growth and metastasis of them to the surrounding tissues. Hypoxia and signaling pathway of HIF are related to the embryonic development and pathology of many human diseases. The role of these two factors in increasing the amount of oxygen delivery to the cell by angiogenesis and activation of glycolysis is proved.

Hernandez *et al*. (2014) used three different type of mouse to examine the role of HIF-1A in acute myeloid leukemia at the phases of beginning, progress and self-repairing of initial leukemic cells. They unexpectedly couldn't see a delay or prevention in the disease progress from the hematopoietic cell without HIF-1A. In some of the examined models, the removal of HIF-1A led to the fast progress of this disease and leukemia. Therefore, these results need reconsideration in the role of HIF-1A and its general therapy functions in AML (Velasco *et al.,* 2014).

Chen *et al.* (2015) examined the HIF-1 expression on AML and the relation of its expression level with clinical parameters and prognosis. Over-expression of HIF-1a has a close relation with the external influence of bone marrow and may be used as an indicator of the external influence of bone marrow and prognosis. In general, these data present new therapy information and showed the fact that the purpose of putting HIF in leukemia has no effect on the normal hematopoietic cells and the patients suffering from leukemia may be cured.

It was observed that the value of gene expression has no relation with patient's gender and age and the P value was insignificant. In this study, the average of genes expressions in patients were obtained by ΔΔCt. The average expression of miR-199b in patients was 2.08 and the average of the normal sample was 0.91; 2.28 times increase of expression was seen and P value was significant (P-value < 0.0001) which showed the significant relation of increase in expression of this gene and the disease.

Talia *et al*. (2014) in their study used three different types of mice to investigate the role of HIF-1 in acute myeloid leukemia in the beginning, development, and self-construction stages of Leukemic Initial Cells (LICs). They unexpectedly failed to observe delay or prevent disease progression in hematopoietic stem cells lacking HIF-1A. In some examined models, removing the HIF-1A accelerated the progression of the disease and increased the severity of blood cancer. Therefore, these results postulate to reconsider the role of HIF-1 and make the general therapeutic applications in AML uncertain.

Vukovic *et al*. (2015) examined the impact of the genetic removal of HIF-2α or both HIF-1α and HIF-2α on different stages of the leukemia production in mice. Results showed that the removal of HIF-2α causes

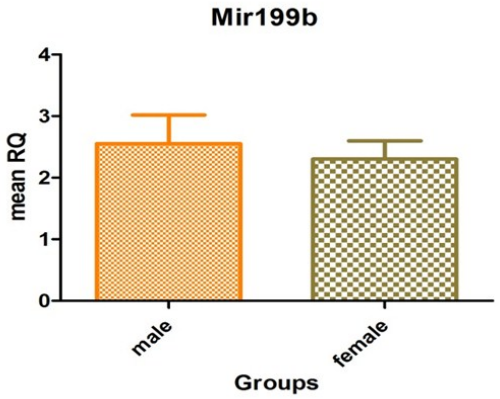


**Figure 8. Gender comparison**

the faster spread of the leukemic stem cells but does not affect the AML spread in AML mice induced by Meis1 / Hoxa9 as well as derived from Mll-AF9. They also found out that HIF-1α and HIF-2α expand the AML but is not required for disease proliferation and pharmacological inhibition of HIF pathway has no effect on the survival and proliferation of AML human cells.

## CONCLUSION

According to the results of this research, we conclude that the average of genes expression in patients in HIF-1 was 0.61 by ΔΔCt method which in contrast with normal sample with the average of 0.92, it reduced 1.5 times and P value was <0.0001 and significant which shows a significant relation between increase of expression of this gene with disease.This is the first study conducted in Iran which examines the expression of HIF-1 gene and miR-199b as effective genes in AML with real-time PCR method. It is hoped that more studies be conducted on more people with AML in order to reduce the harms of other treatments and identify and treat the disease with more accuracy and validity.

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