EFFECTIVENESS MIR-93, MIR-20A, MIR-20B, RORC, STAT3, CD4+, SMAD6, SMAD7, MTOR, FOXO1, FOXP3, GATA3, PPARG, INFG, HIF1A, IL-17, IL-23, IL-1R, IL-21R, IL-21, IL-6, T-BET, SMAD4, SMAD2, STAT5A, STAT5B, STAT1, STAT4, STAT6, IN CELL
DIFFERENTIATION NAÏVE CD4+ FROM TH17 CELLS IN PATIENTS WITH MULTIPLE SCLEROSIS IN THE CITY OF TABRIZ IN IRAN

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ABSTRACT
One of the most basic cells of the immune system in multiple sclerosis patients, th17 cells are involved in many other autoimmune diseases, is effective. miRNAs recently used in the treatment of many diseases. miRNAs, non-coding RNAs with a length of 22-25 nucleotides batch of the regulatory role in various cellular processes. In this study, the use of non-coding miRNAs in Th17 cells in patients with multiple sclerosis to evaluate the performance of these cells was conducted.

KEYWORDS: multiple sclerosis, cells, Th17, miRNAs, CD4 + gene RORC, gene STAT3, autoimmune disease.

INTRODUCTION
Multiple sclerosis (MS), also known as disseminated sclerosis or encephalomyelitis disseminata, is a demyelinating disease in which the insulating covers of nerve cells in the brain and spinal cord are damaged. This damage disrupts the ability of parts of the nervous system to communicate, resulting in a wide range of signs and symptoms,[1,2] including physical, mental,[2] and sometimes psychiatric problems.[3] MS takes several forms, with new symptoms either occurring in isolated attacks (relapsing forms) or building up over time (progressive forms).[4] Between attacks, symptoms may disappear completely; however, permanent neurological problems often occur, especially as the disease advances.[4]
While the cause is not clear, the underlying mechanism is thought to be either destruction by the immune system or failure of the myelin-producing cells.\textsuperscript{[5]} Proposed causes for this include genetics and environmental factors such as infections.\textsuperscript{[2,6]} MS is usually diagnosed based on the presenting signs and symptoms and the results of supporting medical tests.

There is no known cure for multiple sclerosis. Treatments attempt to improve function after an attack and prevent new attacks.\textsuperscript{[2]} Medications used to treat MS, while modestly effective, can have adverse effects and be poorly tolerated. Many people pursue alternative treatments, despite a lack of evidence. The long-term outcome is difficult to predict, with good outcomes more often seen in women, those who develop the disease early in life, those with a relapsing course, and those who initially experienced few attacks.\textsuperscript{[7]} Life expectancy is on average 5 to 10 years lower than that of an unaffected population.\textsuperscript{[1]}

Multiple sclerosis is the most common autoimmune disorder affecting the central nervous system.\textsuperscript{[8]} As of 2008, between 2 and 2.5 million people are affected globally with rates varying widely in different regions of the world and among different populations.\textsuperscript{[9]} In 2013, 20,000 people died worldwide from MS, up from 12,000 in 1990.\textsuperscript{[10]} The disease usually begins between the ages of 20 and 50 and is twice as common in women as in men.\textsuperscript{[11]} The name multiple sclerosis refers to scars (sclerae—better known as plaques or lesions) in particular in the white matter of the brain and spinal cord.\textsuperscript{[12]} MS was first described in 1868 by Jean-Martin Charcot.\textsuperscript{[12]} A number of new treatments and diagnostic methods are under development.

Demyelination by MS. The CD68 colored tissue shows several macrophages in the area of the lesion. Original scale 1:100.

Signs and symptoms
A person with MS can have almost any neurological symptom or sign, with autonomic, visual, motor, and sensory problems being the most common. The specific symptoms are determined by the locations of the lesions within the nervous system, and may include loss of sensitivity or changes in sensation such as tingling, pins and needles or numbness, muscle weakness, very pronounced reflexes, muscle spasms, or difficulty in moving; difficulties with coordination and balance (ataxia); problems with speech or swallowing, visual problems (nystagmus, optic neuritis or double vision), feeling tired, acute or chronic pain, and bladder and bowel difficulties, among others. Difficulties thinking and emotional problems such as depression or unstable mood are also common. Uhthoff's phenomenon, a worsening of symptoms due to exposure to higher than usual temperatures, and Lhermitte's sign, an electrical sensation that runs down the back when bending the neck, are particularly characteristic of MS. The main measure of disability and severity is the expanded disability status scale (EDSS), with other measures such as the multiple sclerosis functional composite being increasingly used in research.
The condition begins in 85% of cases as a clinically isolated syndrome over a number of days with 45% having motor or sensory problems, 20% having optic neuritis, and 10% having symptoms related to brainstem dysfunction, while the remaining 25% have more than one of the previous difficulties.\textsuperscript{[16]} The course of symptoms occurs in two main patterns initially: either as episodes of sudden worsening that last a few days to months (called relapses, exacerbations, bouts, attacks, or flare-ups) followed by improvement (85% of cases) or as a gradual worsening over time without periods of recovery (10-15% of cases).\textsuperscript{[11]} A combination of these two patterns may also occur\textsuperscript{[4]} or people may start in a relapsing and remitting course that then becomes progressive later on\textsuperscript{[11]} Relapses are usually not predictable, occurring without warning.\textsuperscript{[1]} Exacerbations rarely occur more frequently than twice per year.\textsuperscript{[1]} Some relapses, however, are preceded by common triggers and they occur more frequently during spring and summer.\textsuperscript{[17]} Similarly, viral infections such as the common cold, influenza, or gastroenteritis increase their risk.\textsuperscript{[1]} Stress may also trigger an attack.\textsuperscript{[18]} Women with MS who become pregnant experience fewer relapses; however, during the first months after delivery the risk increases.\textsuperscript{[1]} Overall, pregnancy does not seem to influence long-term disability.\textsuperscript{[1]} Many events have not been found to affect relapse rates including vaccination, breast feeding,\textsuperscript{[1]} physical trauma,\textsuperscript{[19]} and Uhthoff's phenomenon.\textsuperscript{[17]}

**Causes**

The cause of MS is unknown; however, it is believed to occur as a result of some combination of genetic and environmental factors such as infectious agents.\textsuperscript{[1]} Theories try to combine the data into likely explanations, but none has proved definitive. While there are a number of environmental risk factors and although some are partly modifiable, further research is needed to determine whether their elimination can prevent MS.\textsuperscript{[20]}

**Geography**

MS is more common in people who live farther from the equator, although exceptions exist.\textsuperscript{[1][21]} These exceptions include ethnic groups that are at low risk far from the equator such as the Samis, Amerindians, Canadian Hutterites, New Zealand Māori,\textsuperscript{[22]} and Canada's Inuit,\textsuperscript{[1]} as well as groups that have a relatively high risk close to the equator such as Sardinians,\textsuperscript{[11]} inland Sicilians,\textsuperscript{[23]} Palestinians and Parsis.\textsuperscript{[22]} The cause of this geographical pattern is not clear.\textsuperscript{[11]} While the north-south gradient of incidence is decreasing,\textsuperscript{[21]} as of 2010 it is still present.\textsuperscript{[11]}
MS is more common in regions with northern European populations\cite{1} and the geographic variation may simply reflect the global distribution of these high-risk populations.\cite{11} Decreased sunlight exposure resulting in decreased vitamin D production has also been put forward as an explanation.\cite{24,25,26} A relationship between season of birth and MS lends support to this idea, with fewer people born in the northern hemisphere in November as compared to May being affected later in life.\cite{27} Environmental factors may play a role during childhood, with several studies finding that people who move to a different region of the world before the age of 15 acquire the new region's risk to MS. If migration takes place after age 15, however, the person retains the risk of his home country.\cite{1,20} There is some evidence that the effect of moving may still apply to people older than 15.\cite{1}

**Genetics**

MS is not considered a hereditary disease; however, a number of genetic variations have been shown to increase the risk.\cite{28} The probability is higher in relatives of an affected person, with a greater risk among those more closely related.\cite{2} In identical twins both are affected about 30% of the time, while around 5% for non-identical twins and 2.5% of siblings are affected with a lower percentage of half-siblings.\cite{1,2,29} If both parents are affected the risk in their children is 10 times that of the general population.\cite{11} MS is also more common in some ethnic groups than others.\cite{30}

**HLA region of Chromosome 6. Changes in this area increase the probability of getting MS.**
Specific genes that have been linked with MS include differences in the human leukocyte antigen (HLA) system—a group of genes on chromosome 6 that serves as the major histocompatibility complex (MHC).\cite{1} That changes in the HLA region are related to susceptibility has been known since the 1980s,\cite{31} and additionally this same region has been implicated in the development of other autoimmune diseases such as diabetes type I and systemic lupus erythematosus.\cite{31} The most consistent finding is the association between multiple sclerosis and alleles of the MHC defined as DR15 and DQ6.\cite{1} Other loci have shown a protective effect, such as HLA-C554 and HLA-DRB1*11.\cite{1} Overall, it has been estimated that HLA changes account for between 20 and 60% of the genetic predisposition.\cite{31} Modern genetic methods (genome-wide association studies) have discovered at least twelve other genes outside the HLA locus that modestly increase the probability of MS.\cite{31}

**T helper 17 cell**

T helper 17 cells (T\(_h\)17) are a subset of T helper cells developmentally distinct from T\(_h\)1 and T\(_h\)2 lineages producing interleukin 17 (IL-17).

### DIFFERENTIATION

Transforming growth factor beta (TGF-β), interleukin 6 (IL-6), interleukin 21 (IL-21) and interleukin 23 (IL-23) contribute to T\(_h\)17 formation in mice and humans. Key factors in the
differentiation of Th17 cells are, besides others, the signal transducer and the activator of transcription 3 (Stat3) and the retinoic acid receptor-related orphan receptors gamma (RORγ) and alpha (RORα).[1] The Th17 cells can alter their differentiation program ultimately giving rise to either protective or pro-inflammatory pathogenic cells. The protective and non-pathogenic Th17 cells induced by IL-6 and TGF-β are termed as Treg17 cells. The pathogenic Th17 cells are induced by IL-23 and IL-1β.[2] IL-21, produced by Th17 cells themselves, has also been shown to initiate an alternative route for the activation of Th17 populations.[3] Both interferon gamma (IFNγ) and IL-4, the main stimulators of Th1 and Th2 differentiation, respectively, have been shown to inhibit Th17 differentiation.

Functions
Th17 cells play a role in adaptive immunity protecting the body against pathogens. However, anti-fungal immunity appears to be limited to particular sites with detrimental effects observed.[4] Their main effector cytokines are IL-17A, IL-17F, IL-21, and IL-22.[1] Th17 cells mediate the regression of tumors in mice,[5][6] but were also found to promote tumor formation induced by inflammation of the colon in mice.[7]

Th17 cells, particularly auto-specific Th17 cells, are associated with autoimmune disease such as multiple sclerosis, rheumatoid arthritis, and psoriasis.[1] Th17 overactivation against autoantigen will cause type 3 immune complex and complement-mediated hypersensitivity. Rheumatoid arthritis or Arthus reaction belong to this category.[8] Bone erosion caused by
mature osteoclast cells is common in patients with rheumatoid arthritis. Activated T helper cells such as Th1, Th2, and Th17 are found in the synovial cavity during the time of inflammation due to rheumatoid arthritis. The known mechanisms associated with the differentiation of osteoclast precursors into mature osteoclasts involve the signaling molecules produced by immune-associated cells, as well as the direct cell to cell contact of osteoblasts and osteoclast precursors. However, it has been suggested that Th17 can also play a more major role in osteoclast differentiation via cell to cell contact with osteoclast precursors.\textsuperscript{9,10} Th17 cells may contribute to the development of late phase asthmatic response due to its increases in gene expression relative to Treg cells.\textsuperscript{11}

**CD4**

In molecular biology, CD4 (cluster of differentiation 4) is a glycoprotein found on the surface of immune cells such as T helper cells, monocytes, macrophages, and dendritic cells. It was discovered in the late 1970s and was originally known as leu-3 and T4 (after the OKT4 monoclonal antibody that reacted with it) before being named CD4 in 1984.\textsuperscript{2} In humans, the CD4 protein is encoded by the *CD4* gene.\textsuperscript{3,4}

CD4+ T helper cells are white blood cells that are an essential part of the human immune system. They are often referred to as CD4 cells, T-helper cells or T4 cells. They are called
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helper cells because one of their main roles is to send signals to other types of immune cells, including CD8 killer cells, which then destroy the infectious particle. If CD4 cells become depleted, for example in untreated HIV infection, or following immune suppression prior to a transplant, the body is left vulnerable to a wide range of infections that it would otherwise have been able to fight.

STRUCTURE

Schematic representation of CD4 receptor.

Like many cell surface receptors/markers, CD4 is a member of the immunoglobulin superfamily.

It has four immunoglobulin domains (D₁ to D₄) that are exposed on the extracellular surface of the cell:
- D₁ and D₃ resemble immunoglobulin variable (IgV) domains.
- D₂ and D₄ resemble immunoglobulin constant (IgC) domains.

CD4 uses its D₁ domain to interact with the β₂-domain of MHC class II molecules. T cells expressing CD4 molecules (and not CD8) on their surface, therefore, are specific for antigens presented by MHC II and not by MHC class I (they are MHC class II-restricted). MHC class I contains Beta-2 microglobulin The short cytoplasmic/intracellular tail (C) of CD4 contains a special sequence of amino acids that allow it to interact with the lck molecule.

FUNCTION

CD4 is a co-receptor that assists the T cell receptor (TCR) in communicating with an antigen-presenting cell. Using its intracellular domain, CD4 amplifies the signal generated by the TCR by recruiting an enzyme, the tyrosine kinase Lck, which is essential for activating many molecular components of the signaling cascade of an activated T cell. Various types of T helper cells are thereby produced. CD4 also interacts directly with MHC class II molecules on the surface of the antigen-presenting cell using its extracellular domain. The extracellular domain adopts an immunoglobulin-like beta-sandwich with seven strands in 2 beta sheets, in
a Greek key topology. During antigen presentation, both the TCR complex and CD4 are recruited to bind to different regions of the MHCII molecule (α1/β1 and β2, respectively). Close proximity between the TCR complex and CD4 in this situation means the Lck kinase bound to the cytoplasmic tail of CD4 is able to tyrosine-phosphorylate the Immunoreceptor tyrosine activation motifs (ITAM) present on the cytoplasmic domains of CD3. Phosphorylated ITAM motifs on CD3 recruits and activates SH2 domain-containing protein tyrosine kinases (PTK) such as Zap70 to further mediate downstream signal transduction via tyrosine phosphorylation, leading to transcription factor activation including NF-κB and consequent T cell activation.

RAR-related orphan receptor gamma

RAR-related orphan receptor gamma (RORγ) is a protein that in humans is encoded by the RORC (RAR-related orphan receptor C) gene. RORγ is member of the nuclear receptor family of transcription factors.
GENE EXPRESSION

Two isoforms are produced from the same RORC gene,\textsuperscript{[3]} probably by selection of alternative promoters.\textsuperscript{[4][5]}
• RORγ (also referred to as RORγ1) – produced from an mRNA containing exons 1 to 11.\(^6\)

• RORγt (also known as RORγ2) – produced from an mRNA identical to that of RORγ, except that the two 5’-most exons are replaced by an alternative exon, located downstream in the gene. This causes a different, shorter N-terminus.\(^4\)

**RORγ**

The mRNA of the first isoform, RORγ is expressed in many tissues, including thymus, lung, liver, kidney, muscle, and brown fat.\(^2\)\(^7\)\(^8\) While RORγ mRNA is abundantly expressed, attempts to detect RORγ protein have not been successful therefore it is not clear whether RORγ protein is actually expressed.\(^9\) Consistent with this, the main phenotypes identified in RORγ-/- knockout mice (where neither isoform is expressed) are those associated with RORγt immune system function\(^10\) and an isoform specific RORγt knockout displayed a phenotype identical to the RORγ-/- knockout.\(^10\) On the other hand, circadian phenotypes of RORγ-/- mice\(^11\) in tissues where the RORγt isoform is expressed in minute amounts argues for the expression of functional RORγ isoform. Absent protein in previous studies may be due to the high amplitude circadian rhythm of expression of this isoform in some tissues.

The mRNA is expressed in various peripheral tissues, either in a circadian fashion (e.g., in the liver and kidney) or constitutively (e.g., in the muscle).\(^{12,13}\)

In contrast to other ROR genes, the RORC gene is not expressed in the central nervous system.

**RORγt**

The tissue distribution of the second isoform, RORγt, appears to be highly restricted to the thymus\(^4\) where it is expressed exclusively in immature CD4+CD8+ thymocytes and in lymphoid tissue inducer (LTi) cells.\(^{10}\)\(^{14}\)\(^{15}\) RORγt inhibitors are under development for the treatment of autoimmune diseases such as psoriasis and rheumatoid arthritis.\(^{9,16}\)

**FUNCTION**

The RORγ protein is a DNA-binding transcription factor and is a member of the NR1 subfamily of nuclear receptors.\(^{17}\) Although the specific functions of this nuclear receptor have not been fully characterized yet, some roles emerge from the literature on the mouse gene.
The RORγ isoform appears to be involved in the regulation of circadian rhythms. This protein can bind to and activate the promoter of the ARNTL (BMAL1) gene, a transcription factor central to the generation of physiological circadian rhythms. Also, since the levels of RORγ are rhythmic in some tissues (liver, kidney), it has been proposed to impose a circadian pattern of expression on a number of clock-controlled genes, for example the cell cycle regulator p21.

RORγt is the most studied of the two isoforms. Its best understood functionality is in the immune system. The transcription factor is essential for lymphoid organogenesis, in particular lymph nodes and Peyer's patches, but not the spleen. RORγt also plays an important regulatory role in thymopoiesis, by reducing apoptosis of thymocytes and promoting thymocyte differentiation into pro-inflammatory T helper 17 (Th17) cells. It also plays a role in inhibiting apoptosis of undifferentiated T cells and promoting their differentiation into Th17 cells, possibly by down regulating the expression of Fas ligand and IL2, respectively.

Despite the pro-inflammatory role of RORγt in the thymus, it is expressed in a T<sub>reg</sub> cell subpopulation in the colon, and is induced by symbiotic microflora. Abrogation of the gene's activity generally increases type 2 cytokines and may make mice more vulnerable to oxazolone-induced colitis.

**TBX21**

T-box transcription factor TBX21 is a protein that in humans is encoded by the *TBX21* gene.
This gene is a member of a phylogenetically conserved family of genes that share a common DNA-binding domain, the T-box. T-box genes encode transcription factors involved in the regulation of developmental processes. This gene is the human ortholog of mouse Tbx21/Tbet gene. Studies in mouse show that Tbx21 protein is a Th1 cell-specific transcription factor that controls the expression of the hallmark Th1 cytokine, interferon-gamma (IFNG). Expression of the human ortholog also correlates with IFNG expression in Th1 and natural killer cells, suggesting a role for this gene in initiating Th1 lineage development from naive Th precursor cells.[1]

MATERIALS AND METHODS
In this study, 53 patients with MS and 60 healthy controls were studied. Peripheral blood samples from patients and parents with written permission control was prepared. After separation of serum, using RT-PCR technique of RNA molecules were collected. To isolate cells, CD4 +, erythrocytes were precipitated from hydroxyethyl starch (HES) was used. At this stage, HES solution in a ratio of 1 to 5 with the peripheral blood of patients and controls were mixed. After 60 minutes of incubation at room temperature, the supernatant was removed and centrifuged for 10 min at 300G era. The cell sediment with PBS (phosphate buffered saline), Pipetazh and slowly soluble carbohydrate ratio of 1 to 2 on ficole (Ficol) was poured in the 480G was centrifuged for 24 minutes. Mononuclear cells to CD4 + cells also are included, has a lower density than ficole and so on which they are based. The remaining erythrocytes has a molecular weight greater than ficole and deposited in test tubes. The supernatant, which contained the mononuclear cells was removed, and the 300G era was centrifuged for 12 minutes. Finally, the sediment cell, the antibody and CD4 + was added after 34 minutes incubation at 5 ° C, the cell mixture was passed from pillar LSMACS. Then the cells were washed with PBS and attached to the column LSMACS Spam Stem cell culture medium containing the transcription factors STAT3 RORC and were kept.
Figure 1: Schematic picture of nuclear magnetic resonance system at the bottom of the cells, T-bet, RORC, Th17.

**Flow Cytometry**

To determine the purity of CD4 + cells are extracted, flow cytometry was used. For this purpose, approximately 4-5 × 10³ CD4 + cells were transferred to 1.5ml Eppendorf tube and then was centrifuged at 2000 rpm for 7 minutes at a time. Remove the supernatant culture medium and the remaining sediment, 100μl of PBS buffer was added. After adding 5-10μl CD4-PE monoclonal antibody to the cell suspension for 60 min at 4 °C, incubated and read immediately by flow cytometry. For example, rather than control antibody CD4-PE, IgG1 negative control solution was used.
Figure 2: Schematic view of the extent of CD-4 and CD-8 cells CD 27 in memory of the nervous system, multiple sclerosis.

RNA extraction

Total RNA extraction procedure includes

a) 1ml solution spilled Qiazol on cells, and slowly and carefully mixed and incubated at room temperature for 5 minutes. Then 200μl chloroform solution to target mix, then transfer the micro tubes were added, and the shaker well was mixed for 15 seconds. The present mix for 4 minutes at room temperature and then incubated for 20 min at 4 °C and was centrifuged at 13200 rpm era. Remove the upper phase product were transferred to a new microtube and to the 1 times the volume of cold ethanol was added. The resulting mixture for 24 hours at -20 °C were incubated.b) Then for 45 min at 4 °C and was centrifuged at 12000 rpm era. Remove the supernatant and the white precipitate, 1ml of cold 75% ethanol was added to separate the sediment from micro tubes were vortex well. The resulting mixture for 20 min at 4 °C and by the time we were centrifuged 12000 rpm. Ethanol and the sediment was removed and placed at room temperature until completely dry deposition. The precipitate was dissolved in 20μl sterile water and at a later stage, the concentration of extracted RNA was determined.
RT-PCR analysis
To check the quality of miRNAs, the RT-PCR technique was used. The cDNA synthesis in reverse transcription reaction (RT) kit (Fermentas K1622) and 1μl oligo primers 18 (dT) was performed. Following the PCR reaction 2μM dNTP, 1μg cDNA, Fermentas PCR buffer 1X, 0 / 75μM MgCl2, 1.25 U / μL Tag DNA at 95 °C for 4 min, 95 °C for 30 s, annealing temperature 58 °C for 30 s, and 72 °C for 30 seconds, 35 cycles were performed. Then 1.5% agarose gel, the PCR product was dumped in wells after electrophoresis with ethidium bromide staining and color were evaluated.

Figure 4: Schematic view of image flow cytometry cells ,IL-17A,IL-22,RORC,IFN-γ,TNF-α,IgG in patients with multiple sclerosis versus normal controls.
Using the data obtained, the positive and negative regulators of their role in the differentiation of Th17 cells was proven mechanism of action of these cells was evaluated. Genes RORC, STAT3, IL-17, MTOR and Smad6 / 7 led to the differentiation of Th17 cells, and the categories were positive regulators, and the genes of IFN-γ, Smad3 / 4, GATA3, and T-bet to differentiate into other cell types paths T such as Th1, Th2 or Treg launched, they were as negative regulators of the route. Using bioinformatics database miRWalk, Myanksh miRNA-mRNA was precisely through genetic algorithm. With studies on genes, mRNAs that positive and negative regulators of cell differentiation of Th17 seeds and their interaction with selected miRNAs associated with Th17 cells in autoimmune disease multiple sclerosis was conducted, it was observed that these miRNAs in MS patients influence. The miRNAs affect the disease MS, miR-93, miR-20a and miR-20b respectively. These miRNAs can inhibit positive regulators in the differentiation of CD4 + cells, Th17, in this way disrupt and prevent the increase in the number of these cells and thus reduce inflammation. It should be noted that all 3 of them this precursor miRNAs miR-17 are part of the family. MiR-17 family factors in non-coding called miR-106b, miR-25, miR-20a that miR-20a is a has-miR-17-92 cluster and an important role in the immune system and autoimmune diseases.

Figure 5: Schematic view of bioinformatics and population genetics miR-20a in patients with multiple sclerosis versus normal controls in different periods of life.

Bioinformatics analysis showed that, miR-20a / b and miR-93 inhibition effect through positive regulators, Th17 cell differentiation are doing and therefore inhibit Th17
differentiation pathway. The purpose of the miRNAs are positive regulators include: STAT3, HIF1a, SMAD6, SMAD7 and RORC are, among which, RORC and STAT3 transcription factor in the differentiation of Th17 are one. These factors can also, on mRNA genes, such as: IL-1R, IL-23R and that of other regulators RUNX1 positive, CD4 + baker to Th17 cells are also affected. In addition, miR-20a and miR-20b that can interact with much less power on some of the negative regulators, Th17 cell differentiation, such as: SMAD2 / 4, T-bet and FOXO1 also be affected.

Table 1: The power and potential of miRNA-mRNA interactions based on bioinformatics database miRWalk.

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<td>Has-miR-93</td>
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<td>miRNA</td>
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Figure 6: schematic diagram of the frequency of cells, RORγt, FOXP3, T-bet, GATA3, IL-17, IL-10, IL-4, IFNγ, STAT3-P, STAT1-P, STAT5-P, STAT6-P, STAT4-P In patients with multiple sclerosis.
DISCUSSION AND CONCLUSION

Studies show that these forecasts will Bioinformatics, in this study correspond well with studies that have been conducted. Using information obtained from the mechanism the miRNAs involved in immune cells in peripheral blood of patients and controls, to compare these three miRNAs in the arrangements of positive and negative CD4 + cells and shift them to Th17 cells were studied and the results for the principle that the data of the RT-PCR reaction for the patient and control samples, were significant. In fact, patients with multiple sclerosis affected by epigenetic and genetic echo, it will show the changes in miRNAs. The doctor Cox and his colleagues in 2010 showed that activity, miR-20a, miR-20b and miR-93 in peripheral blood of patients with multiple sclerosis, reduced and can inhibit genes involved in the activation of T cells are. The doctor Steiner and his colleagues in 2011 showed that the activity of miRNA-20a and miRNA-93 in cells T, have a positive effect.

Figure 7: Schematic view of the frequency of CD4 cells in the nervous system memory to run patients with multiple sclerosis.
CONCLUSIONS RESEARCH

miRNAs studied in this research, can be used as a biomarker to detect early-stage disease and multiple sclerosis patients before the onset of clinical symptoms in infected person used. Thus, a significant reduction of the miRNAs in the blood, it can be a biomarker for the diagnosis of disease severity or activity of Th17 cells. According to these results, it can be argued that, miR-93, miR-20a, miR-20b by inhibiting STAT3 transcription factors such as RORC and Th17 differentiation to prevent and can be used as medicinal and therapeutic potential and also identify biomarkers for Multiple Sclerosis use.

The results of the PCR reaction

Figure 8: Schematic view of the pattern formed stat3 band.
Figure 9: Frequency Distribution miR-20a, miR-20b, CDKN1A with nucleotide sequences in MS patients versus controls.

Figure 10: Schematic view of information processing system for cell flow cytometry, Th17 pathogenic and Non-pathogenic Patients with MS.
Figure 11: Schematic view of the nucleotide sequence mir-17 and mir-20a with two nucleotide difference.

Figure 12: Schematic view of codons forming SMAD4 with variety miRNAs.
Figure 13: Schematic view of the structure miR-20b in MS patients in a control group under an electron microscope.
Figure 14: Schematic view of the nucleotide sequence of the DNA with methylation pattern bond formation with MS patients and a control group of unmethylation.

Table 2: The most important distinction between the positive and negative regulators to Th17. The regulators in several cellular pathways leading to differentiation of Th17 cells differentiate into the cell or block.

<table>
<thead>
<tr>
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<th>Positive regulators</th>
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<td>IFN-γ</td>
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<td>IL-6</td>
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<td>IL-21</td>
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<td>PPARg</td>
<td>IL-22</td>
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REFERENCES


