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A Comparative Study Of Mirna 146 Is An Identification Of Inflammation Related To The Bone Formation, And Regulation In Prolonged Asthma And Chronic Obstructive Pulmonary Diseases

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ABSTRACT:

MiRNAs are upcoming identifiable bio- marker in various disease prognostic diagnostic and therapeutic approaches for various diseases. Respiratory diseases like asthma and COPD can trigger at any time by the exposure of dust, allergens like pollen, pets or emotional upsets. The condition it may increase the financial burden and prolonged use of medication cause further decreasing the immunity may worsen the situation. The study hypothesis is that, prier identification of disease status to prevent further disease progression. Material and methods: total 40 serum samples were collected after receiving the informed consent from the 15-stable asthma, 15 stable COPD and from 10 controls from normal individuals without having any other infectious and complicated diseases like diabetes, liver, renal, cardiovascular, or any other endocrinal disturbances. Results: Serum samples were assessed by rt-pcr, and statistical analysis were performed. High expression of MiRNA 146 in case of COPD. Comparison Procedures (Bonferroni t-test): got significance with COPD verses controls, COPD verses asthma, non-significance with asthma verses controls. Conclusion: The differential diagnosis of various stages of disease were ruled out by identifying the serum levels of MiRNA 146a. Further detection of treatment effect on disease state get benefited by avoid use of more dosage of inhaled corticosteroids and its adverse effect on other systems like skeletal, dermal and endocrine disturbances.

KEYWORDS: Micro RNA, bone mineral density, inhaled corticosteroids, osteoporosis and osteopenia.

INTRODUCTION:

Double standard endogenous RNA called as Micro RNA (MiRNA) interfering the genetic expression[1]. Disturbances in regulation of miRNAs and their effects in various diseases have been demonstrated in various studies. By altering gene expression, miRNAs may alter the cellular activities such as proliferation, apoptosis, differentiation, and migration in multiple types of diseases including renal diseases, cardiovascular dysfunctions, lung diseases, and cancers[2 - 6].

Types of research areas focus on the development of miRNA therapeutics for the treatment of an extensive range of human diseases. Few disease treatments like hepatitis C virus (HCV) infection, the first-ever miRNA therapeutic is in phase II clinical trials. miravirsen is for miR-122 and is rapidly moving[7] LNAs are novel nucleic acid analogues with some therapeutic value. Basically, they are changed or customized RNA nucleotide introducing intravenously.

Coming to the present asthma In 2016, Panganiban et al. did a differential miRNA profile among asthmatics, non-asthmatics with allergic rhinitis, and non-asthmatic non-allergic subjects[8]. In their study, the researchers found 30 miRNAs in plasma that were differentially expressed among three groups, showing six miRNAs (miR-125b, miR-16, miR-299-5p, miR-126, miR-206, and miR-133b) with a high predictive value when differentiating allergic and asthmatic status. Moreover, some of these circulating miRNAs grouped asthmatic patients into two clusters according to the number of peripheral blood eosinophils. Finally, they demonstrated that circulating miRNAs could be used to diagnose both allergic rhinitis and asthmatic patients and characterize asthma subtypes.

Milger et al., in 2017, identified some possible plasma miRNA candidates as biomarkers in a murine model of asthma[9]. These miRNAs were validated in a different cohort of healthy subjects and asthmatic patients, using a regularized logistic regression model to identify five miRNA ratios that are able to differentiate allergic asthmatics from controls with an area under the curve (AUC) of 0.92. However, this miRNA signature did not differentiate asthma sub-phenotypes.

miRNA-based treatment has emerged as a potential approach for clinical intervention in some respiratory diseases such as asthma and COPD. It is based on miRNAs delivery in the specific site of action which constitutes one of the main aspects of development in relation to miRNA like therapeutic approach. A long list of miRNAs has been found to be linked to initiation, progression, or exacerbations in both respiratory diseases, especially in COPD. However, some have been studied more in depth, showing a high potential as future therapeutic tools through their up- or downregulation. In this sense, miR-146a [10,11], miR-21 [12,13], miR-150 , miR[14]-145-5p [15,16], miR-320d [17,18], miR-155 [19]miR-223[20] or miR-3162-3p [21]seem to hold promise as future elements in the therapeutic repertoire for COPD and asthma, respectively, some of which are common for both pathologies such as miR-146a[22-24] or miR-21 [25,26].

Material and methods:

With the stable conditioned routine follow-up for checking the disease state of 15asthma, 15COPD and 10 controls were selected for the analysis of MiRNA146a.

Ethical committee approval and Collected informed consent from all the subjects.

Methodology

RNA isolation and quantitative RT-PCR.

Total RNA in serum from all participants was extracted using mi RN easy (Qiagen, Valencia, CA). RNA quality and concentration were assessed by spectrophotometry. miR-146a were assayed using Taqman miRNA assays (Life Technologies) according to the manufacturer's protocol. About 40 ng of total RNA and quantified using miRNA standard curves. For mRNAs, total RNAs were reverse transcribed with Reverse Transcription System kits (Promega, USA) and amplified using Power Up SYBR Green Master Mix (Applied Biosystems, USA).

MicroRNA-Seq

MicroRNA-Seq library preparation and sequencing was performed by IGA Technology Services (Udine, Italy) using the Illumina Tru Seq Small RNA Library Preparation kits (Illumina, San Diego, USA), according to manufacturer instructions. Overexpression of miR-146a was accomplished by amplifying a genomic fragment of ~280 bp,

The following oligonucleotides were used in semiquantitative RT-PCR approach:

Table No: 1 The Primer Sequences Used For The PCR Amplification Were As Follows:

Genes	Forward primer	Reverse Primer	Amplicon size (bps)
miR-146a	5'-CACGGACCTGAAGAACACTGG -3'	5'-AGAAATGAAATTAGAACACACAT -3'	280
snRNA U6	5'-GAGTGACCAGGCCCCTTGTCT-3'	5'-TTGCTCTTTTCACTCTCATTCTGGTGA-3'	564

Each PCR mix contained 10 µl TaqMan universal PCR master mix 1 µl 20x TaqMan micro RNA assay, 1.33 µl template cDNA and 7.76 µl nuclease free water to complete the reaction mixture to 20 µl. The prepared reaction mixtures were processed in the Applied Biosystems Step One thermal cycler. The cycling conditions were as follows: Annealing was carried out at 60˚C for 30 s, extension at 72˚C for 30 s, and denaturation at 95˚C for 15 s for 40 cycles. Quantitative analysis of miR-146a expression was determined using SYBR Premix Ex TaqTM II (Ta Ka Ra, Japan). Levels of miR-146a were calculated utilizing the $2^{-\Delta\Delta t}$ method with U6 as the internal reference.

Results: Data analyzed by mean, SD mentioned in table no – 2,one way ANNOVA and pair wise multiple Comparison Procedures (Bonferroni t-test) done. Got significant P value with the comparison of asthma verses COPD, COPD verses controls and non - significance with control verses asthma.

Table no 2 shows the comparative expression levels of controls, asthma and COPD cases

Table 2 : Comparative effect of MiRNA 146 expression of control, asthma and chronic obstructive pulmonary disease (COPD).

S.No.	Variable	Category	Control	Asthma	COPD	Statistical analysis
1	MiRNA Expression	Mean	1.520	1.614	2.667	F = 30.882
		SD	0.239	0.253	0.615	P < 0.001
2	Gender	Male	4	8	10	$\chi 2 = 1.751$
		Female	6	7	5	P = 0.417
3	Age	< 40 years	2	3	3	$\chi 2 = 0$
		> 40 years	8	12	12	P = 1
n – Control = 10; Asthma = 15; COPD = 15.						

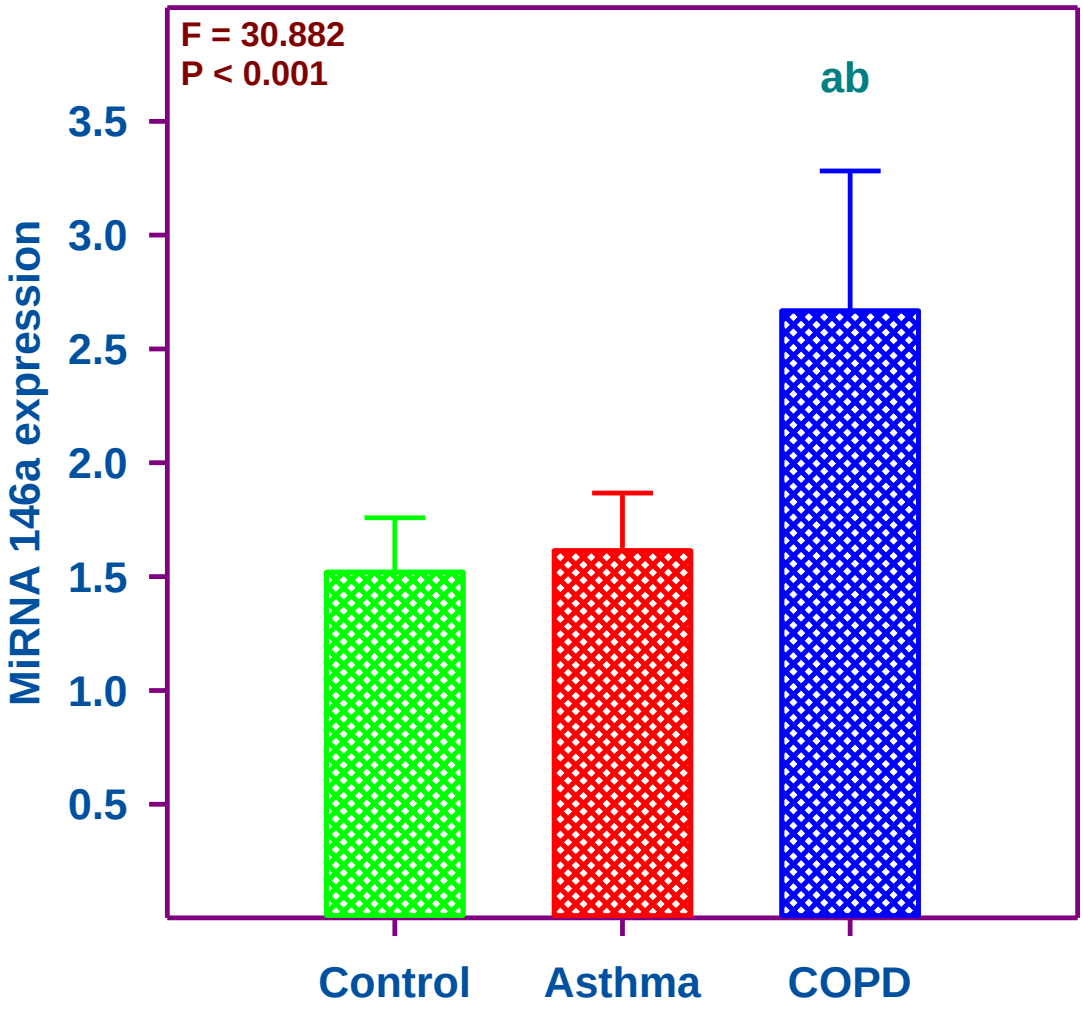

Figure x.x: Comparative effect of MiRNA 146a expression of control, asthma and chronic obstructive pulmonary disease (COPD).

Values are mean ± SD (n – Control = 10; Asthma = 15; COPD = 15).

The 'F' and 'P' values are by one way ANOVA with Bonferroni 't' test.

[a]Significantly different from control group.

[b]Significantly different from asthma group.

DISCUSION: increased proinflammatory cytokines like IL – 4, IL – 5, IL – 9 and IL – 13 and decreased anti-inflammatory cytokines lead to cause asthma[20]. Based on the predominant immunological pathway, two distinguishable phenotypes of asthma have been identified: Th2 asthma and non-Th2 asthma. In Th2 asthma, patients show an excellent response to corticosteroids,

whereas non-Th2 asthma is more liable to exhibit resistance to conventional corticosteroid therapy[21].

In MSCs derived from periodontal ligament tissue (PDLSCs) overexpression of miR-21 was correlated with decreased expression of alkaline phosphatase (ALP), as well as Runx-2. The study showed that miR-21 decreased osteogenic potential of PDLSCs by targeting Smad5 molecule, a component of BMPs signaling pathway that is activated during osteoblastogenesis. Furthermore, Wei et al. showed that transfection of cells with miR-21 inhibitor stimulated the osteogenic differentiation of h PDLSCs and improved mineralization of ECM [22]. The role of miR-21 has been studied also in bone resorbing cells (osteoclasts). Suppression of miR-21 was associated with upregulation of osteoclast suppressor programmed cell death protein 4 (PDCD4), and downregulation of osteoclast marker cathepsin K (CTSK) [23]. Thus, miR-21 may be involved in bone biology, not only via promoting mobilization of osteoblast precursors, but also by regulation of osteoclast survival and differentiation [24]. It was also shown that miR-21 knockout mice are characterized by normal skeletal phenotype during development and maintain osteoblastogenesis in vivo. However, miR-21-knockout mice showed increased expression of receptor activator of nuclear factor κB ligand (RANKL) accompanied by decreased level of osteoprotegerin (OPG). Both molecules are major osteoblastic mediators of osteoclastogenesis. RANKL is an essential cytokine promoting differentiation and maturation of osteoclasts, while OPG acts as a decoy receptor for RANKL. OPG inhibits osteoclast differentiation by blocking the interaction between RANKL and RANK,

Present study included stable cases without any complication of other diseases. As a result, by giving proper dosage with an indication of serum 146 levels may avoid the side effect of medication. In case of if treated with large doses also may get treated by introducing miR- 21 as a therapeutic option

CONCLUSION: Assessment of serum micro RNA analysis in controls and subjects gave an idea of disease maintenance. Those who are on proper treatment got near by values like normal subjects, that means the treatment is working in those persons. Initial stage of disease with slight symptoms can be proven by analyzing the micro RNA 146 as a prognostic approach. In severe gold stage V COPD persons, to get the condition of drug resistance to avoid the unnecessary side effect of an inhaled corticosteroids.

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