Circulating miRNA-146a and miRNA-146b-5p in psoriasis and in response to psoriasis therapy

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Abstract

MiRNA-146a is overexpressed in psoriasis. MiRNA-146a and miRNA-146b have partially overlapping functions. We evaluated blood miRNA-146a/b expression by syber green real time-PCR in psoriasis and in response to mono-therapy using narrow-band ultraviolet B (NB-UVB) phototherapy or methotrexate (MTX), and evaluated if they are possible biomarkers for disease activity and for response to NB-UVB and MTX therapies.. MiRNA-146a/b expression significantly increased in psoriasis patients before therapy than in the controls. However, miRNA-146a expression showed significantly higher fold increase than miRNA-146b. Moreover, miRNA-146a/b expression levels before therapy were significantly higher in the MTX group than in the NB-UVB group, which coincided with the higher PASI score in the MTX group before therapy. In addition, miRNA-146a/b expression significantly decreased in response to both therapies. The fold decrease after MTX therapy relative to before MTX therapy was significantly higher than that after UVB therapy relative to before UVB therapy, which coincided with the significantly higher reduction effect of MTX on the PASI score. Only miRNA-146a significantly and positively correlated with PASI score. In conclusion, miRNA146a/b expression levels are increased in psoriasis and decreased in response to psoriasis therapy and may be used in predicting the response to NB-UVB therapy or methotrexate. Also, miRNA-146a may be used as a biomarker for disease activity.

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INTRODUCTION

Psoriasis is considered a chronic inflammatory immune mediated skin disease, with an overall prevalence of 2–3% of the world's population [1], It is characterized by relapsing skin lesions displaying exaggerated and disordered epidermal cell proliferation and keratinization, an inflammatory infiltrate in the dermis and epidermis and angiogenesis [2,3].

Many environmental factors as physical trauma, infections, stress and drugs can all trigger an initial episode of psoriasis in those individuals with genetic predisposition [4]. In genetically predisposed individuals, on exposure of the epidermis to microbial or mechanical injury, interaction of damage associated molecular patterns (DAMP) / pathogen associated molecular patterns (PAMP) with their receptors, such as Toll like receptors (TLRs) and nucleotide-binding oligomerization domain receptors (NOD like receptors; NLRs), causes the activation of keratinocytes and the epidermal innate immune system, leading to liberation of inflammatory cytokines as tumour necrosis factor (TNF)-α, interleukin (IL) IL-1β and IL-6. These cytokines are potent chemoattractants that recruit antigen presenting cells (as macrophages and specific dendritic cells (DCs); myeloid DCs with TLRs and plasmacytoid DCs) in the skin dermis. Once the APCs engulf the inciting antigen, they migrate to the skin draining lymph nodes where they secrete TNF-α, interferon (IFN)-γ, IL-12 and IL- 23 to activate the naïve CD4+ T cell into T helper (Th) 1, Th 17 and Th22 cells. Th cells migrate into the lesional skin where they produce cytokines and chemokines that amplify the inflammation and

drive epidermal hyperproliferation, aberrant keratinocyte differentiation and vascular hyperplasia [2,3,5].

MicroRNAs (miRNAs) are small non-coding RNAs that can play critical roles as regulators of many pathways and biological processes including the innate and adaptive immune responses and inflammation [6,7].

MiRNA-146 was one of the first miRNAs identified to be involved in the regulation of immune functions [8]. In the human genome, the miRNA-146 family consists of two genes: miRNA-146a on chromosome 5 and miRNA-146b on chromosome 10. The mature form of miRNA-146a and miRNA-146b only differ by two nucleotides in the 3` end, a region thought to have only a compensatory role for target recognition [6,8]. It is likely that miRNA-146a and miRNA-146b have partially overlapping functions, but their regulation is different and the differences in their physiological roles still need to be determined [6]. MiRNA-146a was found to be overexpressed in the psoriatic skin lesions [9,10,11] and in the peripheral blood monocytes (PBMCs) of psoriatic patients [10].

A recent important implication of miRNAs in the clinical medical field is their ability to determine the efficacy of drugs [12]. Narrow-band ultraviolet B (NB-UVB) phototherapy is commonly used in the treatment (ttt) of psoriasis [13]. It is considered highly effective in extensive psoriasis. At a rate of three sessions per week, it results in complete or almost complete lesions eradication in 60 to 90 % of patients within 20 to 40 sessions [14]. NB-UVB phototherapy is accompanied by suppression of type I and type II

IFN signaling, down modulation of the Th17 pathway and modulation of genes involved in epidermal differentiation in lesional psoriatic epidermis. In addition, several anti-inflammatory pathways as vitamin D, peroxisome proliferator activated receptor, glucocorticoid and IL-4 signaling, are modulated by NB-UVB therapy [15].

MTX is a drug that competitively inhibits dihydrofolate reductase enzyme and several other folate dependent enzymes resulting in inhibition of thymidylate and purine synthesis and thus inhibition of DNA and RNA synthesis. Nucleic acid synthesis inhibition in activated T-cells and in keratinocytes is believed to account for the anti-proliferative and immune-modulatory effects of MTX which are considered the main mechanisms of the therapeutic effect of MTX in psoriasis [16].

This study was designed to evaluate the effect of psoriasis and anti-psoriasis therapies, including NB-UVB phototherapy and MTX therapy, on the gene expression levels of miRNA-146a/b in the blood and to evaluate if they are possible biomarkers for disease activity and for response to NB-UVB and MTX therapies. This is the first study to report the changes in miRNA-146b expression and its relation to miRNA-146a expression in psoriasis and in response to NB-UVB and MTX therapies.

MATERIALS AND METHODS

Subjects

The study was conducted on 30 patients with moderate or severe chronic plaque psoriasis determined by the psoriasis area and severity index (PASI) score. The patients were selected from the outpatient clinic of dermatology, Benha University Hospitals. Fifteen age and gender matched healthy volunteers with no family history of psoriasis or any systemic autoimmune diseases were included as a control group.

The patients group was divided into 2 subgroups according to the therapy they received. The UVB group; included 15 patients who received NB-UVB therapy. The irradiation was administered to the whole body two to three times a week with an initial dose of 0.1–0.3 J/cm2 per session, increased accordingly according to the skin tolerance and clinical response. The MTX group; included 15 patients who received methotrexate 12.5 mg as single weekly Intramuscular dose.

All patients included in the study did not receive phototherapy or systemic immunosuppressive treatment for at least 4 weeks or even topical therapy for 2 weeks before inclusion in the study. Smokers, alcoholics, diabetics, patients with renal or hepatic disease, and pregnant or lactating females were excluded from the study.

All the study subjects or their parents gave written informed consent. Also, the ethical committee of the Faculty of medicine, Benha University approved the protocol of the study.

Relative quantitation of miRNA-146a/b

- *Blood Sampling*

Two blood samples (1 ml each) were withdrawn from each psoriasis patient one before and another 8 weeks after the initiation of therapy with NB-UVB irradiation or MTX. Fifteen additional blood samples were drawn from the healthy volunteers of control group. 100μL of each

sample was collected in into RNA Protect Animal Blood tube, Qiagen-Germany. The tubes were left to stand for 2 hours before being stored at -80°C for further processing.

- *Separation of WBCs and total RNA Extraction Including miRNA*

At first, separation of the White blood cells was done according to the manufacturer instructions of RNeasy® Protect Animal Blood kit (Qiagen, GmbH Hilden, Germany). The 100 µL of blood in the RNA Protect Animal Blood tube was transferred to eppendorrf tube and centrifuged for 3 mins at 6500rpm. The supernatant was removed and 1 ml of RNAse free water was added to the pellet then vortexed until the pellet was visibly dissolved. The number of cells was read on Automated Hematology Analyzer, Sysmex-Japan. Then the tube was centrifuged for 3 mins at 6500 rpm and all the supernatant was removed. Later the cell pellet was loosened by flickering of the tube, then according to the manufacturer instructions of miRNeasy mini kit (Qiagen, GmbH Hilden, Germany), 700 µL QIAzol lysis reagent was added and the remaining steps of RNA extraction were proceeded. Extracted RNA was evaluated for purity and quantity using nanodrop 2000 (Thermoscientific, USA).

- *Reverse Transcription Step*

MiRNAs and the house keeping gene snRNA U6 were reverse transcribed into cDNA using specific reverse transcription (RT) primers as follows: hsa-miRNA-146a stem loop primer: GTCGTATCCAGTGCGTGTCGTGGAGTCGGC AATTGCACTGGATACGACaacccca; hsa-miRNA -146b-5p stem loop primer: GTCGTATCCAG TGCGTGTCGTGGAGTCGGCAATTGCACTGG

ATACGACagccta; U6: CGCTTCACGAATTTG CGTGTCAT [17]. The reaction mixture included 25 ng total RNA, 1 μL of RT enzyme, 2 μL of RT buffer, 0.5 μL (20 units) RNase inhibitor, 2 μL of the specific primer, 2 μL of dNTPs, , and nuclease-free water up to 20 μL. The cDNA was then diluted to 200 μL.

- *Real Time PCR for relative quantitation of miRNA-146a and miRNA-146b-5p*

It was performed using super real premix plus (sybr green) kit which is supplied by (TIANGEN, Biotech, Beijing). The sequences of the primers used were: hsa-miRNA-146a: GGGTGAGAAC TGAATTCCA; hsa-miRNA-146b-5p: GGGTG AGAACTGAATTCCA; the universal reverse PCR primer: CAGTGCGTGTCGTGGAGT; U6-forward: GCTTCGGCAGCACATATACTAAAA T and U6-reverse: CGCTTCACGAATTTGCGT GTCAT [17]. The reaction mix contained 10 μL 2x super real premix plus, 0.5 μL of forward primer, 0.5 μL of reverse primer, 5 μL of cDNA, 0.4 50x ROX reference dye, and 3.6 RNase-free water. Amplification of miRNA-146a and U6 were done in separate PCR tubes. PCR was performed in step one real time PCR (Applied Biosystems, Singapore). The optimized thermal profile involved initial denaturation at 95°C for 15 min followed by 40 cycles of 95°C for 10 sec., 54°C for 1 min., 72°C for 30 sec. and a final extension at 72°C for 30 sec. Melting curve was done to ensure the specificity of the reaction.

Relative expression of microRNA of interest was calculated as ΔCt, measured by subtracting the Ct of the target miRNA from that of the snRNA U6. Lower ΔCt values indicated higher expression level of miRNA. Fold expression changes were

calculated using the equation $2^{-\Delta\Delta Ct}$ [18]. When comparing the study groups relative to the control group, the mean ΔCt of the control group was used as a calibrator. In addition, when comparing the fold change of MTX group before therapy relative to UVB group before therapy, the mean ΔCt of the UVB group before therapy was used as a calibrator. However, on comparing UVB group or MTX group after therapy to before therapy, ΔCt of individual samples of UVB and MTX groups before therapy were used as calibrators respectively.

The laboratory work was conducted in the Molecular Biology Unit, Faculty of Medicine- Benha University, while the quantitation of cells was done in the Chemical Endocrinology unit, Faculty of Medicine- Benha University.

Statistical Analysis

The study data were computed and statistically analyzed using Statistical Package for the Social Science (SPSS) version 23 software. Quantitative variables were expressed as mean ± SD, mean±SE or median and inter quartile range (IQR). Parametric data were compared using student *t*-test, paired student *t*- test or anova test where appropriate. Non parametric data were compared by Mann-Whitney U-test (for unpaired data) or Wilcoxon signed rank test (for paired data). Qualitative variables were expressed as number and percentage and compared by chi square test. Spearmen correlations were done for correlating different variables. In all tests, p value was considered significant if less than 0.05.

RESULTS

This study was conducted on 45 participants stratified into: 1. UVB Group; including 15

chronic plaque psoriasis patients who received NB-UVB therapy, 2. MTX group; including 15 chronic plaque psoriasis patients who received MTX therapy, 3. Control group; including 15 age and gender-matched healthy volunteers. Relative quantitation of blood miRNA-146a and 146b-5p was done in all the participants before and 8 weeks after therapy in the UVB and MTX groups. Some important demographic parameters are presented in table (1).

MiRNA-146a blood levels

Analysis of the mean±SE of fold change in the blood expression level of miRNA-146a in psoriasis patients relative to the normal controls revealed that miRNA-146a displayed significant fold increase in expression of 106.9±44.3 in psoriasis patients before UVB, of 289.2±144.3 before MTX and of 179.8±64.9 in all patients before therapy in relation to the control group (p<0.05, p= 0.001 and p< 0.001 respectively). After therapy, miRNA-146a expression level decreased but continued to show significant fold increase relative to the control group (after UVB; 40.7±21.9, after MTX; 96.7±76.6 and in all patients after therapy; 63.1±32.5) with p<0.05 (Fig.1A). Moreover, blood miRNA-146a expression level showed significant fold increase of 8.9±2.4 in the MTX group before therapy relative to the UVB group before therapy (p<0.05) (Fig.1B).

Analysis of mean±SE of fold change in the blood expression level of miRNA-146a in psoriasis patients after UVB therapy relative to before UVB therapy revealed significant 2.9 fold decrease with fold change of 0.34±0.47 in the psoriasis patients after UVB (p<0.05). Moreover,

Table (1): Some demographic parameters of the studied participants

Parameter	Control group N=15	UVB group N=15	MTX group N=15
Age Mean±SD	43.87±12.2	43.47±12.22	42.53±9.7 *
Gender • Female (Number/ %) • Male (Number/ %)	(6/ 40%) (9/60%)	(8/53.3%) (7/46.7%)	(7/46.7%) * (8/53.3%)
PASI score • **Before Therapy** (Mean±SD) Median (IQR) • **After therapy** (Mean±SD) Median(IQR)	----- -----	14.33±4.24 13 (3) 8.56±2.6 [#] 8 (2)	30.5±4.9 ** 29.5(9) 15.67±3.2 **[#] 15(6.5)
• **Percentage of reduction of PASI score after therapy (%)** (Mean±SD)	-----	40.33±4.25	48.82±3.6 **

* P>0.05 is non significant, ******P <0.001 is significant, [#] P<0.001 is significant when after therapy compared to before therapy,.

the mean±SE of fold change of miRNA-146a level in psoriasis patients after MTX therapy relative to before MTX therapy was 0.21±0.07 indicating 4.8 fold decrease of miRNA-146a level after MTX therapy which was significant (p<0.05) (Fig.1C). The fold decrease after MTX therapy relative to before MTX therapy *(4.8 fold decrease)* was significantly higher than that after UVB therapy relative to before UVB therapy *(2.9 fold decrease)* (p<0.05).

MiRNA-146b blood levels

Analysis of mean±SE fold change in the blood expression level of miRNA-146b in psoriasis patients before therapy relative to the normal control revealed a significant fold increase results. Mean±SE fold increases before therapy were 15.3±4.3 (p<0.05), 57.4±8.3 (p<.001), 25.8±6.1 (p<0.05) in the UVB group, MTX group and all patients respectively. After therapy, miRNA-146b expression levels decreased to non significant levels relative to the control group (p>0.05). The

mean±SE fold change in psoriasis patients after UVB was 3±1.1, after MTX was 5.2±1.07 and in all patients after therapy was 4.1±0.8 (Fig. 2A). In addition, there was significant fold increase of blood miRNA-146b expression level in the MTX group before therapy relative to the UVB group before therapy with mean±SE fold change of 13.04±1.9 (p=0.001). (Fig.2B).

Analysis of mean±SE fold change in the blood expression level of miRNA-146b in psoriasis patients after UVB therapy relative to before UVB therapy revealed a significant 7.2 fold decrease (p<0.001) with mean±SE fold change of 0.138±0.016 in the psoriasis patients after UVB. Moreover, the mean±SE fold change of miRNA-146b level in psoriasis patients after MTX therapy relative to before MTX therapy was 0.079±0.01 indicating 12.7 fold decrease of miRNA-146b level after MTX therapy which was significant (p<0.001) (Fig.2C). The fold decrease after MTX

therapy relative to before MTX therapy *(12.7 fold decrease)* was significantly higher than that after UVB therapy relative to before UVB therapy *(7.2 fold decrease)* (p<0.05).

Comparison between miRNA-146a and miRNA-146b levels before therapy

MiRNA-146a expression showed significantly higher fold increase than miRNA-146b expression in the blood of psoriatic patients before therapy compared to the normal control (p= 0.001), (Figures 1A, 2A).

Correlation of miRNA-146a and miRNA-146b with some demographic data and each other

The study revealed that there was non significant correlation between miRNA-146a level and age, sex among all patients. However, miRNA-146a showed positive significant correlation with PASI score. On the other hand, miRNA-146b showed non significant correlation with age, sex and PASI score. Moreover, miRNA-146a level showed non significant positive correlation with miRNA-146b level (Table 2).

Table 2: Correlation of miRNA-146a and miRNA-146b with demographic data and each other

Variables	rho
MiRNA-146a:	
age	0.33*
sex	0.03*
PASI score	0.47**
MiRNA-146b	0.36*
MiRNA-146b:	
age	0.16*
sex	0.05*
PASI score	0.17*

* P> 0.05,   ** P< 0.05

DISCUSSION

Psoriasis can be initiated by infection in genetically predisposed individuals [4]. The innate immune response, mediated by epithelial and immune cells as macrophages and dendritic cells, is the first line of defense against infection. Commonly, this response is mediated through activation of members of the Toll/interleukin-1 receptor (TIR) superfamily, which are divided into two groups; interleukin-1 receptors (IL-1Rs) and TLRs. The IL-1Rs family mediate the responses to IL-1α, IL-1β and IL-18, while the TLRs family are involved in the recognition of PAMPs of the invading microorganisms [19,20]. All receptors of this superfamily are thought to signal through a similar intracellular pathway involving IL-1R associated kinase 1 (IRAK1) recruitment which on dissociation, activates TNF receptor-associated factor 6 (TRAF6) leading to the activation of a variety of pro-inflammatory transcription factors as nuclear factor (NF)-κB and activator protein (AP)-1. To prevent an inappropriate inflammatory response that may occur following activation of the TIR receptors, a variety of extracellular and intracellular negative feedback pathways occur to regulate this process [21].

It was found that miRNA-146a/b expression can be induced in response to LPS-induced TLR -2, -4 or -5 activation in the myeloid cells by bacterial and fungal pathogens, or in response to proinflammatory
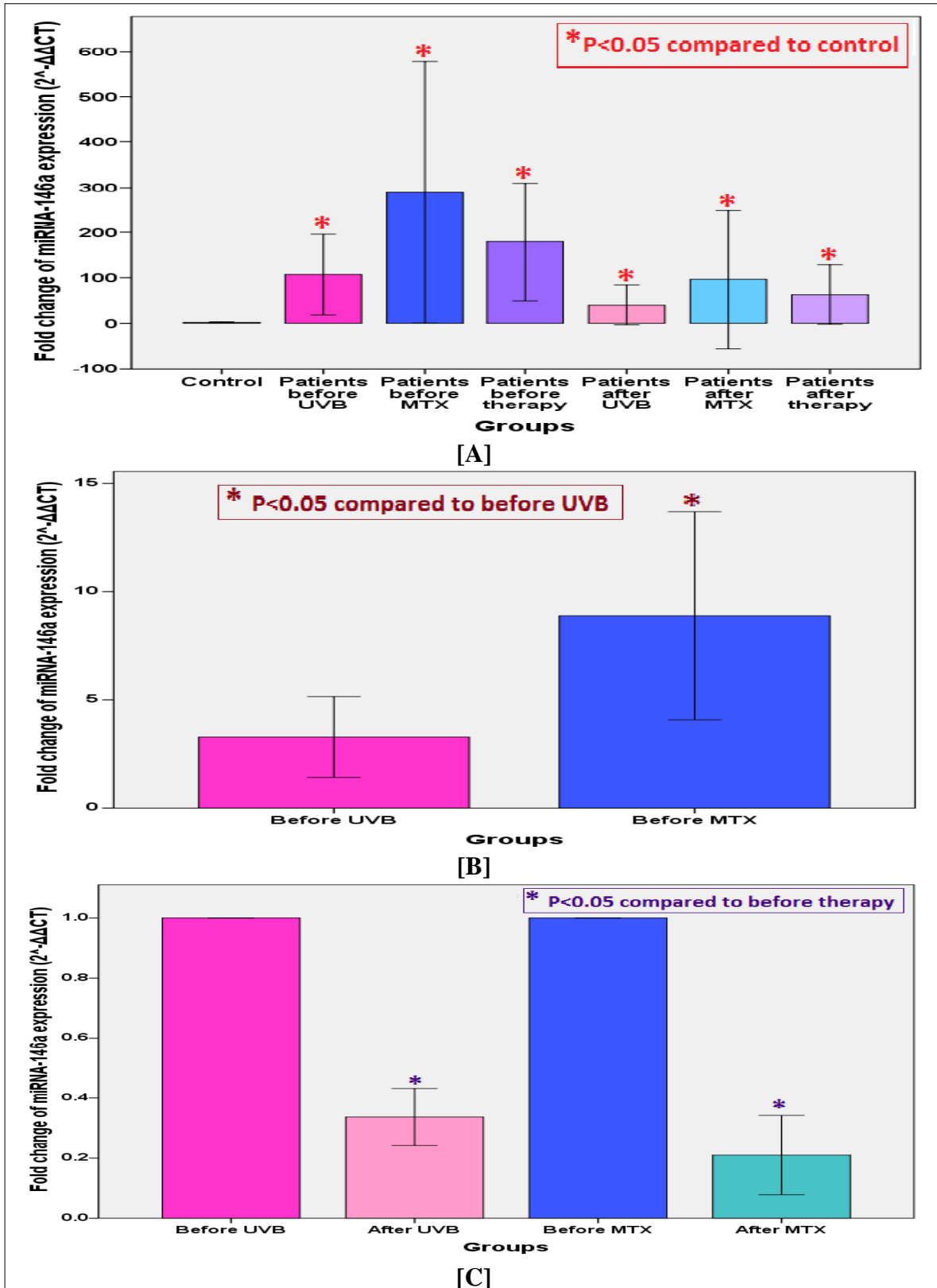
Figure (1): Showing fold changes in the expression level of blood miRNA-146a.
A) In the different studied groups compared to the control group.
B) In the MTX group before therapy compared to the UVB group before therapy.
C) In the UVB group after therapy compared to before therapy and in the MTX group after therapy compared to before therapy.
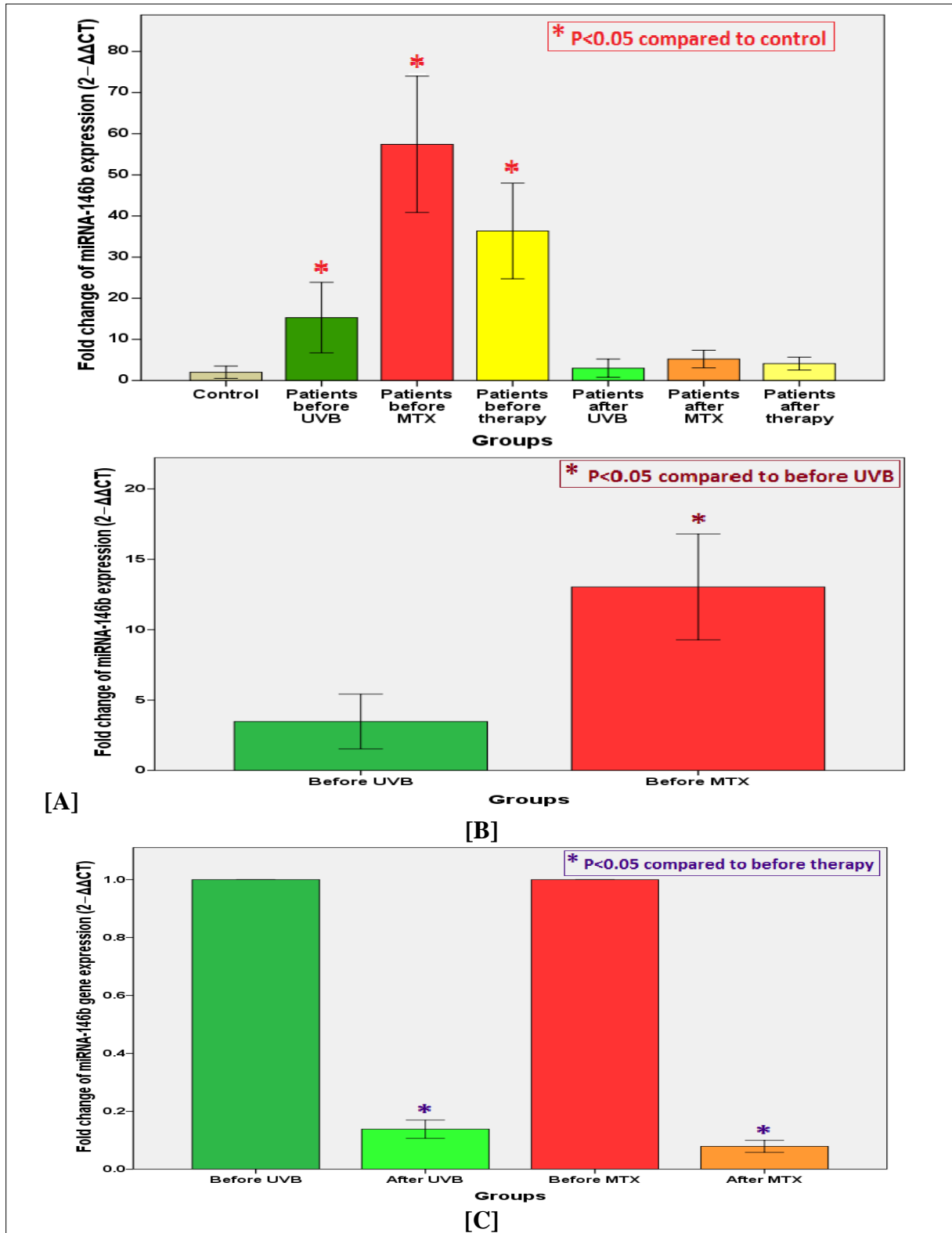Data are presented as mean±SE.

Figure (2): Showing fold changes in the expression level of blood miRNA-146b in :
  A)   The different studied groups compared to the control group.
  B)   The MTX group before therapy compared to the UVB group before therapy.
  C)   The UVB group after therapy compared to before therapy and in the MTX group after therapy compared to before therapy.
  Data are presented as mean±SE.

cytokines such as TNFα or IL-1β [8,22,23,24]. Moreover, activation of TLRs of the Myeloid cells by LPS, and liberation of IL-1 β and TNF- α, induced by microbial or mechanical injury to the skin are included in the pathways involved in psoriasis pathogenesis [3]. This may explain the increase in miRNA-146a and miRNA-146b-5p expression in the blood of psoriatic patients compared to the normal control that was revealed in the current study. Similarly, previous studies reported that miRNA-146a gene expression was significantly higher in the peripheral blood monocytes (PBMCs) of psoriatic patients compared to the healthy controls [12] and in the psoriatic skin lesions than in healthy skin or non lesional skin of psoriatic patients [10,11,12].

The induction of miRNA-146a by LPS-induced TLR -2, -4 or -5 activation in the myeloid cells by pathogens, or by proinflammatory cytokines such as TNFα or IL-1β was found to be through nuclear factor kappa (NF-kB)-dependent mechanisms [8,24]. Also, it was suggested that miRNA-146b is induced also through NF-kB-dependent mechanisms [24]. MiRNA-146a/b produce negative feedback inhibition of the activation of NF-kB and AP-1 pathways by downregulating IRAK1 and TRAF6. This plays a role in fine-tuning innate immune responses in order to decrease the inflammation [8]. In addition to IRAK1 and TRAF6 genes, IRAK2 has been identified as

another target gene of miR-146a which regulates IFNγ production. MicroRNA-146a can cause feedback inhibition of IFNγ production in macrophages by targeting TRAF6, IRAK1, and IRAK2 [25].

Kutty et al. (2013) reported that retinal pigment epithelial cells responded to proinflammatory cytokines (IFN-γ + TNF-α + IL-1β) by highly increasing both miR-146a and miR-146b-5p expression. The miR-146a induction was more dependent on IL-1β and the induction of miR-146b-5p was more dependent on IFN-γ. Also, they found that miR-146b-5p expression regulated by IFN-γ, potentially occur via the JAK/STAT pathway [26].

Another way of induction of miRNA-146b expression in psoriasis which may explain our results may be via activation of AP-1 transcription factor. When the platelet derived growth factor (PDGF) via its ligand BB binds to PDGF receptor, it induces c-Fos; a subunit of AP-1 transcription factor, through activation of the known mitogen activated protein kinases (MAPK) pathway. Binding of c-fos to the miRNA-146b promoter, induces miRNA-146b expression [27]. Since the expression of the beta- subtype of PDGF receptors was found to be greatly elevated in the dermis of psoriatic lesions; dermal fibroblasts and dermal blood vessels [28,29], this may explain the elevation of miRNA146b expression in the blood of psoriatic patients compared to the normal control in this study.

The differential expression of PDGF receptors could regulate the increased proliferation of vascular and connective tissue cells observed in psoriasis [28]. It was found that binding of the homodimer PDGF- BB (type of PDGF ligands) to its type-B receptor, mediates the mitogenic and chemotactic effect of PDGF [30].

In the chronic inflammatory disease; psoriasis there is evidence for the pathogenic involvement of several different cell types that normally occur in skin: keratinocytes, fibroblasts, monocyte-derived immunocytes, T lymphocytes, and mast cells [9]. The elevated miRNA-146a/b expression in the blood in this study is likely to reflect that the source of miRNA-146a/b is the skin infiltration by inflammatory cells. This is supported by the already mentioned finding of induction of expression of 146a/b by activation of myeloid cells' TLRs [8]. Also, this is in accordance with the high expression of miRNA-146a in the immune organs containing significant numbers of leukocytes as thymus and spleen and its low expression in healthy skin, which suggest that infiltrating cells express miRNA-146a in the skin. Moreover, miRNA-146a expression, studied in a panel of cells present in healthy and/or inflamed skin, was absent in keratinocytes and dermal fibroblasts, but preferentially present in immune cells. In particular, monocyte-derived dendritic cells (MDDCs), CD4+CD25 high regulatory T cells and mast cells expressed miRNA-146a at a high level [9]. In addition, up regulation of miRNA-146a in this study may be specific for Th1/Th17 type inflammation as miRNA-146a is up regulated in diseases with aTh1/Th17 type inflammation as psoriasis and rheumatoid arthritis [3,12,31], and not up regulated atopic eczema or mild asthma which are diseases with Th2 type inflammation [3,32]. Also higher expression of miRNA-146a in murine was found in Th1 but not Th2 cells [9,33].

In the current study, miRNA-146a expression showed significantly higher fold increase than miRNA-146b expression in the blood of psoriatic patients compared to the normal control. This is in accordance with the observed 30-and 3-fold increase in miRNA-146a and miRNA-146b expression, respectively following LPS stimulation of the human monocytic THP-1 cell line. This is also in agreement with much higher increase in the expression levels of miRNA-146a than miRNA-146b in epithelial cell lines following IL-1β stimulation [24].

The current study reported significantly higher fold increase of blood miRNA-146a/b expression level in the MTX group before therapy relative to the UVB group before therapy, which may be related to significantly higher activity of the disease indicated by significantly higher PASI score in the MTX group before therapy.

The current study revealed significant decrease of miRNA-146a/b expression after NB-UVB therapy or methotrexate. This effect

by NB-UVB therapy may be explained by the induction of T-cell apoptosis which has been shown as the main mechanism by which NB-UVB resolves psoriatic lesions [34,35,36]. In addition, NB-UVB therapy is associated with suppression of type I and type II IFN signaling [15]. There are two different classes of interferons, Type I (alpha, beta, and omega) and Type II (gamma) interferons [37]. IFN-α provides an important signal for the differentiation of CD4$^+$T cells into Th 1 cells. Furthermore, IFN-α is responsible for the amplification of the CD8$^+$ T cell responses and natural killer cell activation [38]. Since CD4$^+$Th 1, CD8$^+$T cells and natural killer cells have been implicated to be important in the pathogenesis of psoriasis [39], and since the source of miRNA-146a in psoriasis was found to be immune cells especially emphasizing CD4$^+$Th 1 [9], this may explain partially why we reported significant decrease of miRNA-146a expression after NB-UVB therapy. Moreover, it was found that interferon alpha-2b decreases the expressions of PDGF BB mRNAs [40], which may partially explain the decrease in miRNA146b expression with NB-UVB therapy. Moreover, the inhibition of type II interferon signaling by NB-UVB therapy [15] may also contribute to the decrease of miRNA-146b, since its expression may be regulated by IFN-γ via the JAK/STAT pathway [26].

Another important explanation for the decrease in miRNA-146a/b expression by NB-UVB therapy may be related to the down modulating effect of this therapy on Th17 pathway [15]. It was found that miRNA146a/b expression was significantly higher in expanded IL-17 producing T cells than in CD4+ T cells [41]. The down expression effect of MTX on miRNA-146a/b may be explained by the inhibition of nucleic acid synthesis in activated T-cells and keratinocytes [16]. By inhibiting DNA synthesis, MTX can limit epithelial hyperplasia, reinforces activated T cells apoptosis, and inhibits neutrophils chemotaxis. In addition, the drug is responsible for decreasing the formation of a range of proinflammatory cytokines as TNF-α and IL-1 [42].

The fold decrease of miRNA-146a/b after MTX therapy relative to before MTX therapy was significantly higher than that after NB-UVB therapy relative to before UVB therapy indicating better efficacy of MTX in the treatment of psoriasis. This is in accordance with the current study finding of significantly higher reduction of PASI score after MTX therapy than after NB-UVB therapy, which was also reported by [43].

There was a significant positive correlation between miRNA-146a expression levels and PASI score. This suggests that miRNA-146a may be important as a biomarker for psoriatic disease activity. However, miRNA-146b did not show such significant correlation, so it cannot be used as a marker of psoriasis

activity. In addition, miRNA-146a/b did not show significant correlation with age and sex. In agreement with our results, Sawada et al. (2014) revealed that circulating miR-146a is not affected by aging or sex differences [44].

CONCLUSION

Blood miRNA-146a/b expression levels are increased by psoriasis and are decreased in response to NB-UVR and MTX. We suggest that blood miRNA-146a expression changes may serve as biomarker for disease activity in psoriasis and that miRNA146a/b may be used in monitoring the response to NB-UVB therapy or methotrexate. Further research is needed to determine the cellular source of miRNA-146b and its possible pathways of activation and actions in psoriasis. Also, further studies on larger numbers of patients are needed.

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