Clinical Rheumatology (2019) 38:877–884
https://doi.org/10.1007/s10067-018-4380-z

ORIGINAL ARTICLE

MicroRNA-132, miR-146a, and miR-155 as potential biomarkers of methotrexate response in patients with rheumatoid arthritis

Ankita Singh[1] · **Pradeepta Sekhar Patro**[1] · **Amita Aggarwal**[1]

Received: 18 August 2018 / Revised: 21 November 2018 / Accepted: 26 November 2018 / Published online: 3 December 2018

Abstract

Introduction Rheumatoid arthritis (RA) patients have high expression levels of hsa-miR-132-3p, hsa-miR-146a-5p, and hsa-miR-155-5p in peripheral blood. We studied if baseline blood levels of these microRNAs (miRNAs) could predict response to methotrexate (MTX).

Methods RA patients (the American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) criteria) with active disease (disease-modifying anti-rheumatic drug (DMARD)–naïve and Disease Activity Score 28 (DAS28) > 3.2) were enrolled. They were treated with MTX by gradually increasing dose up to 25 mg/week. After 4 months, the DAS28 score was calculated and EULAR response was assessed. The hsa-miR-132-3p, hsa-miR-146a-5p, and hsa-miR-155-5p levels were measured by real-time qPCR in whole-blood RNA at baseline and 4 months after therapy, using hsa-let-7a-5p as housekeeping gene. Results are expressed as median (interquartile range).

Results The 94 enrolled patients (81 females) had a median age of 40 (17) years, disease duration of (24) months, and DAS28 4.61 (1.11). After 4 months of therapy, 73 were classified as responders and 21 as non-responders. Baseline levels of all three miRNAs were lower in responders than non-responders, hsa-miR-132-3p (− 8.03 (0.70) versus − 7.47 (0.85), $P < 0.05$), hsa-miR-146a-5p (− 5.11 (0.88) versus − 4.62 (0.90), $P < 0.05$), and hsa-miR-155-5p (− 7.59 (1.07) versus − 7 (0.72), $P = 0.002$). Receiver operating characteristic curve analysis showed that all three miRNAs were also good predictors of MTX response, showing the following values: hsa-miR-132-3p (area under curve (AUC) 0.756, $P < 0.05$), hsa-miR-146a-5p (AUC 0.760, $P < 0.05$), and hsa-miR-155-5p (AUC 0.728, $P = 0.002$).

Conclusion hsa-miR-132-3p, hsa-miR-146a-5p, and hsa-miR-155-5p are potential biomarkers of responsiveness to MTX therapy.

Keywords Biomarker · Disease-modifying anti-rheumatic drug · Methotrexate · MicroRNA · Rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease which can result in permanent joint damage leading to significant morbidity. All RA patients therefore need early treatment with the disease-modifying anti-rheumatic drugs (DMARDs). Methotrexate (MTX) is the most widely used first-line DMARD for RA due to its good efficacy, rapid onset of action, low cost, and ease of administration [1, 2]. However, due to complexity in disease pathogenesis, a variable response to each DMARD is seen in patients [3]. For instance, 30–40% of RA patients may not respond to MTX therapy. Hence, there is a persistent need for reliable and easy-to-assay biomarker(s) which can predict the response to MTX. Multiple factors such as age, gender, disease duration, baseline rheumatoid factor (RF), C-reactive protein (CRP), genetic variants, and serum levels of proteins such as cytokines and anti-citrulline peptide antibodies (ACPA) have been proposed as predictors of response to MTX [1, 4, 5]. However, despite all efforts, these parameters have fallen short of the expectation [6].

MicroRNA (miRNA) is an endogenous, small noncoding RNA that controls gene expression by hybridizing to complementary sequences in the 3′-UTR of target mRNAs, leading to its degradation or translational repression. MicroRNAs are known to affect the development of all facets of the immune

---

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10067-018-4380-z) contains supplementary material, which is available to authorized users.

✉ Amita Aggarwal
aa.amita@gmail.com

[1] Department of Clinical Immunology and Rheumatology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow 226014, India

system, from hematopoiesis to effector functions [7], and are currently being used to predict the response to therapy with biologicals in RA [8, 9].

The expression of miR-132, miR-146a, and miR-155 has been found dysregulated in RA [10]. Anti-inflammatory effect of miR-132 involves abrogation of the expression of p300 transcriptional co-activator, thereby impairing the production of IL-1β, IL-6, and IFN-β [11]. MicroRNA-146a also acts as a suppressor of the inflammatory response by downregulating TRAF6 and IRAK1 genes [12]. MicroRNA-155 promotes the generation of immunoglobulin class–switched plasma cells especially those producing IgG1, and this effect is mediated via Pu.1 [13]. In the activated $CD4^+$ T cells, overexpression of miR-155 promotes T_H1 cell differentiation and T_H17-mediated production of cytokines such as IL-17 and IL-22 [14]. MicroRNA-155 and miR-146a are upregulated in RA patients compared to healthy controls, which is believed to occur due to persistent inflammation and this may play a role in fine-tuning the genes induced by the inflammatory cascades [15].

Since miR-132, miR-146a, and miR-155 are important modulators of the disease pathology in RA and several studies have shown that the levels of these miRNAs are increased in blood cells as compared to healthy controls [10, 16–22], we studied the potential role of these three miRNAs as biomarkers to predict response to MTX treatment. Further, since MTX is an antimetabolite and antifolate drug and it can affect DNA and RNA synthesis [23], we have also assessed its effect on expression of hsa-miR-132-3p, hsa-miR-146a-5p, and hsa-miR-155-5p after 4 months of therapy.

Materials and methods

Patients

RA patients fulfilling the American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) 2010 classification criteria [24] were included in the study. All had active disease with Disease Activity Score at 28 joints (DAS28) > 3.2 [25]. They were either MTX-naïve or had received MTX > 6 months back and had not taken any other DMARD. Patients having contraindications to MTX treatment or pregnant/lactating were excluded from the study. The study was approved by the Institutional Ethics Committee of SGPGIMS and all patients provided a written, informed consent.

At baseline (BL), clinical data about the disease, physician, and patient global assessment, health assessment questionnaire (HAQ), and DAS28 score were assessed. Blood was collected for miRNA analysis. All patients were started on weekly 10 mg MTX which was escalated every 2 weeks by 2.5 mg until they achieved DAS28 < 2.6, or reached a maximum tolerated dose with an upper limit of 25 mg/week. At the end of 4 weeks, besides assessing disease activity, a blood sample ($n = 80$) was drawn again for estimation of miRNA levels. Patients were classified into two groups, responders and non-responders based on EULAR criteria [24].

Isolation of RNA from blood

Blood was collected in EDTA-coated vials (BD vacutainer 5 ml; BD Diagnostics, San Diego) and stored in aliquots (0.25 ml blood in 0.75 ml Trizol, Life Technologies Inc., USA) at − 80 °C. Total RNA was extracted using chloroform-isopropyl alcohol extraction method. In brief, the sample was brought to room temperature (RT) and vortexed for 15 min. The lysed solution was centrifuged (10,000×*g*, 10 min, 4 °C). To the supernatant, 200 μl chloroform was added and mixed for 15 min by inverting the tube and held for 10 min at RT. The tube was centrifuged (12,000×*g*, 15 min, 4 °C) and the aqueous layer was collected and mixed with 500 μl of isopropyl alcohol by gently inverting the tube (10 min). The mixture was centrifuged (12,000×*g*, 10 min, 4 °C) and the pellet was washed twice by adding 75% ethanol (1 ml) followed by centrifugation (7500×*g*, 5 min, 4 °C). Finally, the pellet was air-dried (30 min) and re-dissolved in 30 μl RNAse-free water. Concentration and purity of RNA were determined using NanoDrop spectrophotometer (NanoDrop Inc.,Wilmington, DE, USA).

miRNA quantification using RT-qPCR

For miRNA analysis, 10 ng of total RNA was reverse transcribed to complementary DNA (cDNA) using hsa-miR-132-3p (assay ID 000457), hsa-miR-146a-5p (assay ID 000468), hsa-miR-155-5p (assay ID 002623), and hsa-let-7a-5p (assay ID 000377) specific stem-loop reverse transcription (RT) primers, 100 mM each dNTP, 50 units/μl MultiScribe reverse transcriptase enzyme, RNase inhibitor, and RT buffer (Applied Biosystems, Carlsbad, CA, USA). The reaction mixture (15 μl) was incubated in a thermocycler (Mastercycler PCR system, Eppendorf, USA) at 16 °C for 30 min, 42 °C for 30 min, and 85 °C for 5 min and then maintained at 4 °C.

Quantification of miRNA was done by real-time PCR performed on resulting cDNA via TaqMan miRNA-specific primers and TaqMan Universal PCR Master Mix without UNG (Applied Biosystems, Carlsbad, CA, USA). The stem-loop and specific sequence of miRNAs are as follows:

hsa-miR-132-3p

Stem-loop:
CCGCCCCCGCGUCUCCAGGGCAACCGUGGCUUUCGAUUGUUACUGUGGGAACUGGAGGUAACAGUCUACAGCCAUGGUCGCCCCGCAGCACGCCCACGCGC

Table 1 Characteristics of rheumatoid arthritis patients

Characteristics	Total (*n* = 94)	Responders (*n* = 73)	Non-responders (*n* = 21)
Female	81 (86.17%)	62 (84.94%)	19 (90.48%)
Age in years	40 (17)	40 (15)	45 (22)
Duration of Disease in months	24 (24)	24 (24)	24 (60)
Tender joint count	11 (7)	10 (7)	11 (11)
Swollen joint count	8.50 (6.75)	9 (6)	7 (7)
Health assessment questionnaire	1.50 (0.88)	1.50 (1)	1.51(0.88)
C-reactive protein, mg/dl (CRP)	2.35 (3.28)	2.50 (3.68)	1.67 (3.09)
Disease Activity Score (DAS28-CRP)	4.61(1.11)	4.60 (0.95)	4.85 (1.73)
IgM-rheumatoid factor–positive	80 (85%)	62 (85%)	18 (85.7%)

Mature miRNA sequence:UAACAGUCUACAGCCAUGGUCG

hsa-miR-146a-5p

Stem-loop:

CCGAUGUGUAUCCUCAGCUUUGAGAACUGAAUUCCAUGGGUUGUGUCAGUGUCAGACCUCUGAAAUUCAGUUCUUCAGCUGGGAUAUCUCUGUCAUCGU

Mature miRNA sequence: UGAGAACUGAAUUCCAUGGGUU

hsa-miR-155-5p

Stem-loop:

CUGUUAAUGCUAAUCGUGAUAGGGGUUUUUGCCUCCAACUGACUCCUACAUAUUAGCAUUAACAG

Mature miRNA sequence: UUAAUGCUAAUCGUGAUAGGGGU

hsa-let-7a-5p

hsa-let-7a-5p 1:

Stem-loop:

UGGGAUGAGGUAGUAGGUUGUAUAGUUUUAGGGUCACACCCACCACUGGGAGAUAACUAUACAAUCUACUGUCUUUCCUA

hsa-let-7a-5p 2:

Stem-loop:

AGGUUGAGGUAGUAGGUUGUAUAGUUUAGAAUUACAUCAAGGGAGAUAACUGUACAGCCUCCUAGCUUUCCU

hsa-let-7a-5p 3:

Stem-loop:

GGGUGAGGUAGUAGGUUGUAUAGUUUGGGGCUCUGCCCUGCUAUGGGAUAACUAUACAAUCUACUGUCUUUCCU

Mature miRNA sequence: UGAGGUAGUAGGUUGUAUAGUU

The reaction mixture was prepared according to the manufacturer's protocol and incubated in LightCycler® 480 II Real-Time System (Roche Applied Science, Penzberg, Germany). Expression levels of miRNAs were normalized with respect to hsa-let-7a-5p gene as its expression in peripheral blood mononuclear cell (PBMC) of RA patient was similar to that of healthy control [10]. The values were expressed as ΔCt, i.e., the Ct value of the miRNA gene was subtracted from the Ct value of the housekeeping gene. Fold change calculation (in brief) is as follows: ΔCt value (miRNA Ctmean-hsa let7a 5p Ctmean), ΔΔCt value (follow-up (FU) ΔCt − BL ΔCt), and fold change as 2^−ΔΔCt.

Statistical analysis

Statistical analysis was performed using SPSS 16.0 (SPSS Inc., USA). The difference at baseline levels of miRNAs between responders and non-responders and between baseline and follow-up samples was assessed using the Mann-Whitney *U* test. Correlation of change in DAS28 at 4 months with hsa-miR-132-3p, hsa-miR-146a-5p, and hsa-miR-155-5p levels was done using Spearman's rank correlation. Logistic regression analysis was done to assess the effect of age, gender, RF, DAS28, hsa-miR-132-3p, hsa-miR-146a-5p, and hsa-miR-155-5p on response status of RA patients. Receiver operating characteristic (ROC) curve analyses for blood miRNAs at

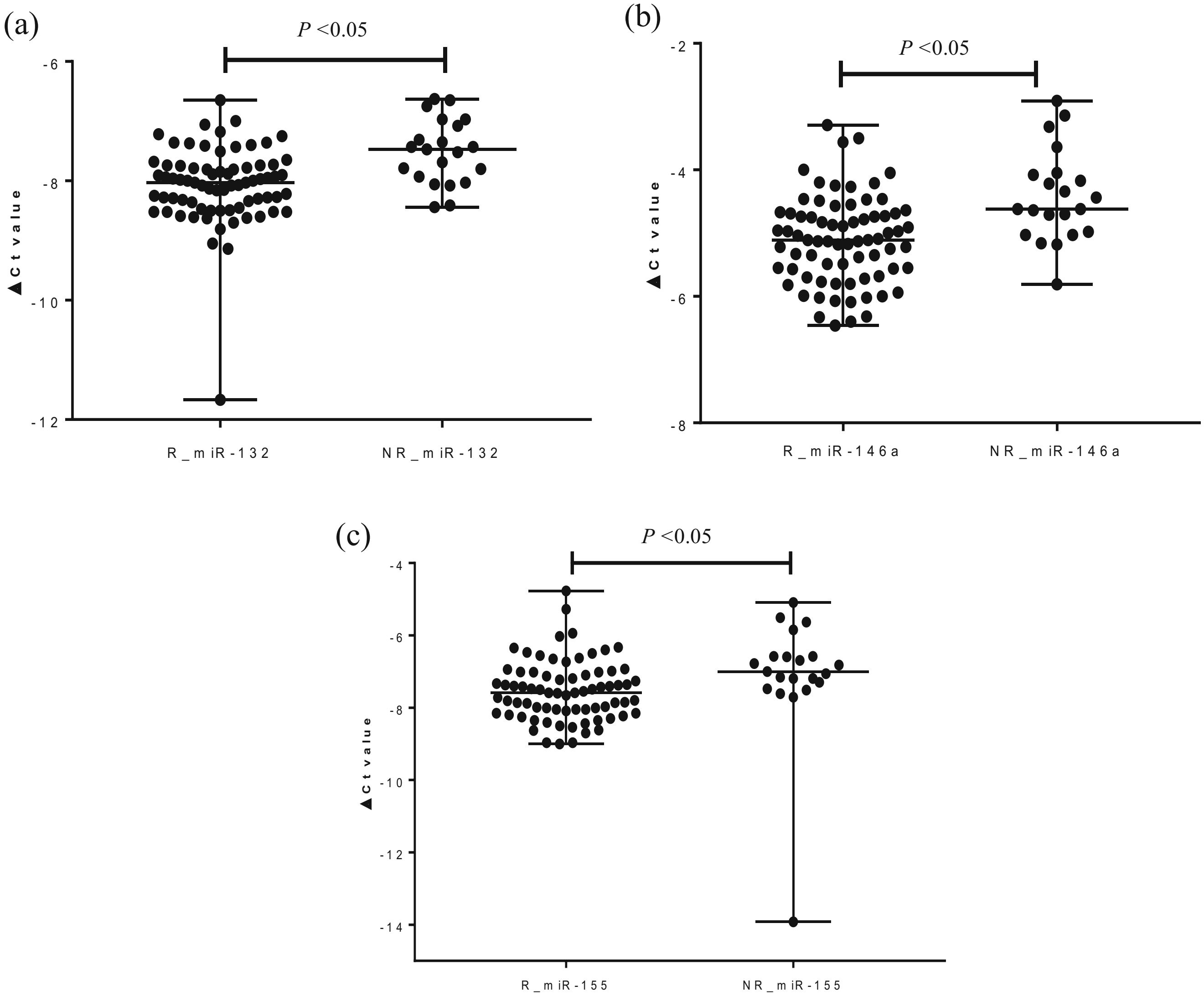

Fig. 1 Baseline expression levels of hsa-miR-132-3p, hsa-miR-146a-5p, and hsa-miR-155-5p in whole blood in responder ($n = 73$) and non-responder ($n = 21$) group of RA patients. **a** hsa-miR-132-3p, **b** hsa-miR-146a-5p, and **c** hsa-miR-155-5p *$P < 0.05$; as determined by the Mann-Whitney *U* test. BL baseline, ΔCt delta threshold cycle, R responder, NR non-responder

baseline was performed and area under the curve (AUC) was calculated. A *P* value less than 0.05 was considered to be statistically significant. All data are expressed as median and IQR.

Results

Patients characteristics

Ninety-four patients (81 females) with a median age of 40 (IQR, 17) years, disease duration of 24 (IQR, 24) months, and DAS28-CRP of 4.61 (IQR, 1.11) were enrolled (Table 1).

Basal levels of miRNA in the blood of patients

The median (IQR) levels of hsa-miR-132-3p, hsa-miR-146a-5p, and hsa-miR-155-5p in 94 patients, respectively, were − 7.96 (0.80), − 4.98 (0.94), and − 7.43 (1.08). There was moderate correlation between hsa-miR-132-3p and hsa-miR-146a-5p ($r = 0.50$, $P < 0.05$), hsa-miR-132-3p and hsa-miR-155-5p ($r = 0.48$, $P < 0.05$), and hsa-miR-146a-5p and hsa-miR-155-5p ($r = 0.65$, $P < 0.05$). No correlation was observed between the levels of hsa-miR-146a-5p or hsa-miR-155-5p with disease activity (as measured by DAS28) while hsa-miR-132-3p showed a minimal correlation ($r = 0.22$, $P = 0.036$). Baseline clinical variables such as RF, erythrocyte sedimentation rate (ESR), CRP, age, gender, and duration of disease did not

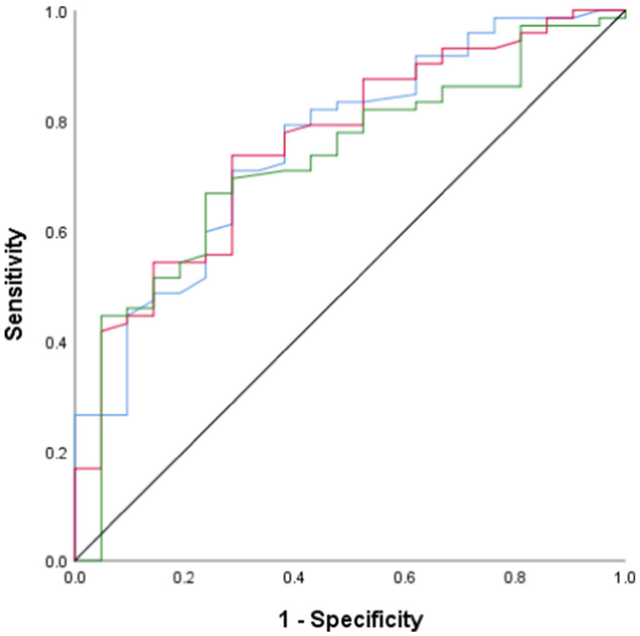

Fig. 2 Receiver operating characteristic (ROC) curve analyses of hsa-miR-132-3p, hsa-miR-146a-5p, and hsa-miR-155-5p as predictors of response to MTX. Blue line depicts hsa-miR-132-3p (AUC 0.76, 95% CI 0.64–0.87), red line is for hsa-miR-146a-5p (AUC 0.76, 95% CI 0.65–0.87), and green line is for hsa-miR-155-5p (AUC 0.73, 95% CI 0.61–0.85)

correlate with the baseline levels of hsa-miR-132-3p, hsa-miR-146a-5p, and hsa-miR-155-5p.

A negative correlation was observed between change in DAS28 score at 4 months (BL DAS28-FU DAS28) with hsa-miR-132-3p ($r = -0.34$, $P = 0.001$) and hsa-miR-146a-5p ($r = -0.30$, $P = 0.004$). A logistic regression analysis was performed to ascertain the effects of age, gender, RF, DAS28, and miRNAs on the likelihood that the patient will respond to MTX therapy. The model correctly classified 86.2% of the cases. Expression levels of two of the miRNAs (hsa-miR-132-3p and hsa-miR-146a-5p) were significantly associated ($P < 0.05$) with the response status of patients (Supplementary Table 1).

Basal levels of miRNA in responders and non-responders for MTX

Among the 94 patients, 73 were responders and 21 non-responders for MTX. In responders, the median levels of hsa-miR-132-3p (− 8.03 (0.70), hsa-miR-146a-5p (− 5.11(0.88), and hsa-miR-155-5p (− 7.59 (1.07) were significantly lower than the corresponding levels in non-responders (− 7.47 (0.85, $P < 0.05$), − 4.62 (0.90, $P < 0.05$), and − 7 (0.72, $P = 0.002$)) (Fig. 1).

On ROC curve analysis, all three miRNAs showed a good AUC: hsa-miR-132-3p (AUC 0.756, 95% CI 0.64–0.87, $P < 0.05$), hsa-miR-146a-5p (AUC 0.760, 95% CI 0.65–0.87, $P < 0.05$), and hsa-miR-155-5p (AUC 0.728, 95% CI 0.61–0.85, $P = 0.002$). Using the cut-off value of − 7.71 for hsa-miR-132-3p, the sensitivity was 79.2% and the specificity was 61.9%. For hsa-miR-146a-5p (cut-off − 4.66), the sensitivity was 78.1% and the specificity was 61.9%, and for hsa-miR-155-5p (cut-off − 7.21), the sensitivity was 69.9% and the specificity was 71.4% (Fig. 2).

Effect of MTX on levels of miRNAs

The expression of hsa-miR-132-3p, hsa-miR-146a-5p, and hsa-miR-155-5p was upregulated after 4 months of MTX therapy (Fig. 3). The changes in relative expression of miRNAs were as follows, hsa-miR-132-3p: fold change 1.56, $P < 0.05$; hsa-miR-146a-5p: fold change 1.31, $P = 0.001$, and hsa-miR-155-5p: fold change 1.61, $P < 0.05$. While the expression of hsa-miR-146-5p (fold change 1.39, $P < 0.001$) and hsa-miR-155-5p (fold change 1.61, $P < 0.05$) increased only in the responder group, the levels of hsa-miR-132-3p increased in both the groups (fold change in responders 1.65 and in non-responders 1.37, $P < 0.05$) (Supplementary Fig. 1).

Discussion

Biomarkers that could predict the response to MTX therapy in RA patients may serve as a valuable tool for early and effective control of the disease. Our study shows that patients who responded to MTX had lower expression of hsa-miR-132-3p, hsa-miR-146a-5p, and hsa-miR-155-5p as compared to those who did not respond to the drug. Though miRNAs have not been studied as a predictor of response to MTX in RA, a recent study has shown plasma miR-27a to be associated with response to a combination therapy of adalimumab with MTX in early RA patients [8]. Another study has shown that serum levels of miR-99a and miR-143 could predict response to adalimumab, whereas miR-197 predicted the response to etanercept [9]. Serum levels of miR-125b could predict the response to rituximab in early RA patients, though its levels in whole blood did not associate with the response [26]. Thus, different miRNAs may predict response to different drugs used in RA.

The association of lower blood levels of these three miRNAs with a good clinical response suggests that their higher levels could have caused dysregulation of the immune response leading to persistent inflammation in non-responders. The combination of anti-TNFα and DMARD therapy has been shown to upregulate the expression of six miRNAs (including miR-146a) in the RA patients who responded to therapy [27].

We did not observe a correlation between levels of hsa-miR-155-5p or hsa-miR-146a-5p and disease activity score, which is in variance to another study that found a positive correlation between miR-146a and disease activity. However, that study had measured miR-146a in peripheral blood mononuclear cells (PBMCs) rather than whole blood [10]. An earlier study using PBMCs had found higher expression of miR-132, miR-146a, and miR-155 in RA patients

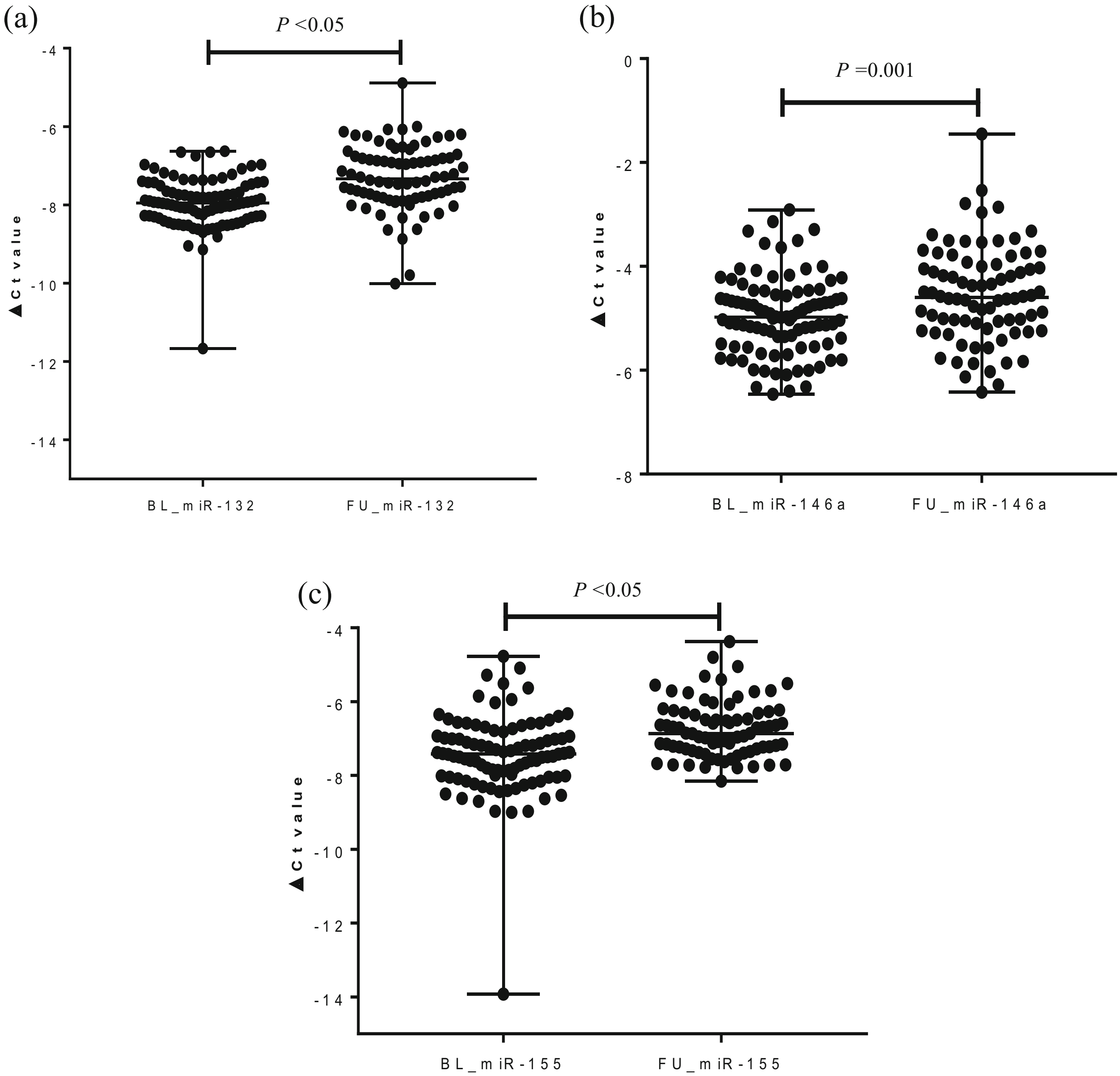

Fig. 3 Expression levels of hsa-miR-132-3p (**a**), hsa-miR-146a-5p (**b**), and hsa-miR-155-5p (**c**) in blood at baseline ($n = 94$) and after 4 months of MTX therapy ($n = 80$). *$P < 0.05$, as determined by the Mann-Whitney U test. BL baseline, FU follow-up, ΔCt delta threshold cycle, R responder, NR non-responder

compared with healthy controls [16]. Another study had used plasma to show overexpression of multiple miRNAs including miR-155 and miR-146a, though the level of miR-132 was found lower than healthy controls [17].

We preferred to use the whole-blood miRNA expression method as it avoids artifacts occurring during isolation of PBMC which can alter miRNA expression levels [28–30]. Though whole-blood miRNA expression profiling has been used in cancer patients [30–32], studies in RA patients are limited. A recent study has compared the levels of miR-146a and miR-155 in PBMC and whole blood and observed that whole-blood miRNA levels accurately reflect those in PBMC [33].

Increased adenosine production is one of the major mechanisms of action of MTX. In turn, adenosine can cause activation of transcription factors CREB and MAPK and induce cAMP production, which can lead to increased expression of miR-132, miR-146a, and miR-155 [34, 35]. MicroRNA-132 and miR-146a suppress inflammation by negatively regulating genes involved in inflammatory pathways, such as MyD88-dependent TLR pathway [34]. As adenosine production is low in non-responders [36], it might account for a lack of difference in the expression levels of miRNAs at the baseline and after 4 months of therapy.

Even though miR-146a counterbalances the function of miR-155 by suppressing the inflammatory response driven by the latter [37], we, paradoxically, observed significant increase in both miRNAs in the MTX non-responders. A study in patients with sepsis has shown that elevated miR-155 expression can induce immunosuppression by CD39 expressing Treg cells and also enhances the Treg cell population [38, 39]. It might be interesting to look for the same correlation in RA where high CD39 expression on Treg is associated with a good response [36]. Moreover, a recent study has also shown that the serum miRNA level (miR-10a) is upregulated after MTX therapy in RA patients [40].

As MTX is the most frequently used drug in RA, multiple predictors of response are being explored. Clinical variables are the easiest and cost-effective as the corresponding data is readily available. Though smoking [41], longer disease duration [42], and higher DAS score at baseline [42] have been associated with poor response to MTX therapy by some studies, others have not found them to be of use. In the present study also, none of the clinical variables like age, gender, DAS score at baseline seropositivity, and duration of disease could predict the response. As genetic markers are more reproducible and reliable, many genes have been identified which could predict response to MTX. For instance, shared epitope–positive RA patients are less likely to respond to MTX monotherapy [43]. There are other studies in which single-nucleotide polymorphism has been shown to predict response to MTX, such as solute carrier family 19 member 1 (*SLC19A1*) [44] and methylenetetrahydrofolate reductase (*MTHFR*) [45] gene polymorphisms. However, the results of genetic studies were not consistently replicated by other groups [6]. In epigenetics, overall methylation and FoxP3 demethylation are induced by MTX in RA patients. Nonetheless, studies on its correlation with response to the drug are still lacking.

In our study, even though clinical variables did not predict the response to MTX, the baseline expression levels of all three miRNAs (hsa-miR-132-3p, hsa-miR-146a-5p, and hsa-miR-155-5p) could do so, albeit with a modest sensitivity and specificity. Therefore, these miRNAs may be considered as additional biomarkers to predict the response to MTX therapy.

One of the limitations of this study is the use of delta Ct between the housekeeping gene and miRNA level. Absolute gene expression analysis using a calibration curve from a standard sample may have better reproducibility across laboratories. To address technical variations to get reproducible results, non-human exogenous controls should be used along with other measures [46]. As this is an exploratory study, the findings need to be validated in another setting in order to assess their true potential.

To conclude, baseline hsa-miR-132-3p, hsa-miR-146a-5p, and hsa-miR-155-5p expression levels in whole blood of RA patients appear to be fair predictors of their response to MTX therapy. The used method requires a much smaller blood sample (than the serum/PBMC-based methods) and is less time-consuming.

Funding statement The project was funded by a research grant to AA and AS was supported by the Senior Research Fellowship of Indian Council of Medical research.

Compliance with ethical standards

Disclosures None.

References

1. Halilova KI, Brown EE, Morgan SL, Bridges SL Jr, Hwang MH, Arnett DK et al (2012) Markers of treatment response to methotrexate in rheumatoid arthritis: where do we stand? Int J Rheumatol 2012:978396
2. Donahue KE, Gartlehner G, Jonas DE, Lux LJ, Thieda P, Jonas BL, Hansen RA, Morgan LC, Lohr KN (2008) Systematic review: comparative effectiveness and harms of disease-modifying medications for rheumatoid arthritis. Ann Intern Med 148:124–134
3. McInnes IB, Schett G (2011) The pathogenesis of rheumatoid arthritis. N Engl J Med 365:2205–2219
4. Fabre S, Dupuy A, Dossat N, Guisset C, Cohen J, Cristol J et al (2008) Protein biochip array technology for cytokine profiling predicts etanercept responsiveness in rheumatoid arthritis. Clin Exp Immunol 153:188–195
5. Van Venrooij WJ, Van Beers JJ, Pruijn GJ (2008) Anti-CCP antibody, a marker for the early detection of rheumatoid arthritis. Ann N Y Acad Sci 1143:268–285
6. Ling S, Bluett J, Barton A (2018) Prediction of response to methotrexate in rheumatoid arthritis. Expert Rev Clin Immunol 14:419–429
7. Davidson-Moncada J, Papavasiliou FN, Tam W (2010) MicroRNAs of the immune system. Ann N Y Acad Sci 1183:183–194
8. Sode J, Krintel SB, Carlsen AL, Hetland ML, Johansen JS, Hørslev-Petersen K et al (2017) Plasma microRNA profiles in patients with early rheumatoid arthritis responding to adalimumab plus methotrexate vs methotrexate alone: a placebo-controlled clinical trial. J Rheumatol 45:53–61
9. Cuppen BV, Rossato M, Fritsch-Stork RD, Concepcion AN, Schenk Y, Bijlsma JW et al (2016) Can baseline serum microRNAs predict response to TNF-alpha inhibitors in rheumatoid arthritis? Arthritis Res Ther 18:189
10. Pauley KM, Satoh M, Chan AL, Bubb MR, Reeves WH, Chan EK (2008) Upregulated miR-146a expression in peripheral blood mononuclear cells from rheumatoid arthritis patients. Arthritis Res Ther 10:R101
11. Lagos D, Pollara G, Henderson S, Gratrix F, Fabani M, Milne RS et al (2010) miR-132 regulates antiviral innate immunity through suppression of the p300 transcriptional co-activator. Nat Cell Biol 12:513–519
12. Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, Guo J, Zhang Y, Chen J, Guo X, Li Q, Li X, Wang W, Zhang Y, Wang J, Jiang X, Xiang Y, Xu C, Zheng P, Zhang J, Li R, Zhang H, Shang X, Gong T, Ning G, Wang J, Zen K, Zhang J, Zhang CY (2008) Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res 18:997–1006
13. Vigorito E, Perks KL, Abreu-Goodger C, Bunting S, Xiang Z, Kohlhaas S, Das PP, Miska EA, Rodriguez A, Bradley A, Smith KGC, Rada C, Enright AJ, Toellner KM, MacLennan ICM, Turner M (2007) microRNA-155 regulates the generation of immunoglobulin class-switched plasma cells. Immunity 27:847–859

14. Zhang J, Cheng Y, Cui W, Li M, Li B, Guo L (2014) MicroRNA-155 modulates Th1 and Th17 cell differentiation and is associated with multiple sclerosis and experimental autoimmune encephalomyelitis. J Neuroimmunol 266:56–63
15. Taganov KD, Boldin MP, Chang K-J, Baltimore D (2006) NF-κB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proc Natl Acad Sci 103:12481–12486
16. Duroux-Richard I, Jorgensen C, Apparailly F (2011) miRNAs and rheumatoid arthritis—promising novel biomarkers. Swiss Med Wkly 141:w13175
17. Murata K, Yoshitomi H, Tanida S, Ishikawa M, Nishitani K, Ito H, Nakamura T (2010) Plasma and synovial fluid microRNAs as potential biomarkers of rheumatoid arthritis and osteoarthritis. Arthritis Res Ther 12:R86
18. Churov AV, Oleinik EK, Knip M (2015) MicroRNAs in rheumatoid arthritis: altered expression and diagnostic potential. Autoimmun Rev 14:1029–1037
19. Pauley KM, Cha S (2011) miRNA-146a in rheumatoid arthritis: a new therapeutic strategy. Immunotherapy 3:829–831
20. Abdul-Maksoud R, Sediq A, Kattaia A, Elsayed W, Ezzeldin N, Abdel Galil S, Ibrahem R (2017) Serum miR-210 and miR-155 expression levels as novel biomarkers for rheumatoid arthritis diagnosis. Br J Biomed Sci 74:209–213
21. Zhou Q, Haupt S, Kreuzer JT, Hammitzsch A, Proft F, Neumann C, Leipe J, Witt M, Schulze-Koops H, Skapenko A (2015) Decreased expression of miR-146a and miR-155 contributes to an abnormal Treg phenotype in patients with rheumatoid arthritis. Ann Rheum Dis 74:1265–1274
22. Su LC, Huang AF, Jia H, Liu Y, Xu WD (2017) Role of micro RNA-155 in rheumatoid arthritis. Int J Rheum Dis 20:1631–1637
23. Rajagopalan PR, Zhang Z, McCourt L, Dwyer M, Benkovic SJ, Hammes GG (2002) Interaction of dihydrofolate reductase with methotrexate: ensemble and single-molecule kinetics. Proc Natl Acad Sci 99:13481–13486
24. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham IIICO et al (2010) 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum 62:2569–2581
25. Prevoo M, Van'T Hof MA, Kuper H, Van Leeuwen M, Van De Putte L, Van Riel P (1995) Modified disease activity scores that include twenty-eight-joint counts development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 38:44–48
26. Duroux-Richard I, Pers Y-M, Fabre S, Ammari M, Baeten D, Cartron G et al (2014) Circulating miRNA-125b is a potential biomarker predicting response to rituximab in rheumatoid arthritis. Mediat Inflamm 2014(23):342524. https://doi.org/10.1155/2014/342524
27. Castro-Villegas C, Pérez-Sánchez C, Escudero A, Filipescu I, Verdu M, Ruiz-Limón P, Aguirre MA, Jiménez-Gomez Y, Font P, Rodriguez-Ariza A, Peinado JR, Collantes-Estévez E, González-Conejero R, Martinez C, Barbarroja N, López-Pedrera C (2015) Circulating miRNAs as potential biomarkers of therapy effectiveness in rheumatoid arthritis patients treated with anti-TNFα. Arthritis Res Ther 17:49
28. Pritchard CC, Kroh E, Wood B, Arroyo JD, Dougherty KJ, Miyaji MM et al (2011) Blood cell origin of circulating microRNAs: a cautionary note for cancer biomarker studies. Cancer Prev Res 5:492–497
29. Kirschner MB, Kao SC, Edelman JJ, Armstrong NJ, Vallely MP, van Zandwijk N, Reid G (2011) Haemolysis during sample preparation alters microRNA content of plasma. PLoS One 6:e24145
30. Häusler S, Keller A, Chandran P, Ziegler K, Zipp K, Heuer S et al (2010) Whole blood-derived miRNA profiles as potential new tools for ovarian cancer screening. Br J Cancer 103:693–700
31. Patnaik SK, Yendamuri S, Kannisto E, Kucharczuk JC, Singhal S, Vachani A (2012) MicroRNA expression profiles of whole blood in lung adenocarcinoma. PLoS One 7:e46045
32. Schrauder MG, Strick R, Schulz-Wendtland R, Strissel PL, Kahmann L, Loehberg CR, Lux MP, Jud SM, Hartmann A, Hein A, Bayer CM, Bani MR, Richter S, Adamietz BR, Wenkel E, Rauh C, Beckmann MW, Fasching PA (2012) Circulating micro-RNAs as potential blood-based markers for early stage breast cancer detection. PLoS One 7:e29770
33. Mookherjee N, El-Gabalawy HS (2013) High degree of correlation between whole blood and PBMC expression levels of miR-155 and miR-146a in healthy controls and rheumatoid arthritis patients. J Immunol Methods 400:106–110
34. Nahid MA, Yao B, Dominguez-Gutierrez PR, Kesavalu L, Satoh M, Chan EK (2012) Regulation of TLR2-mediated tolerance and cross-tolerance through IRAK4 modulation by miR-132 and miR-212. J Immunol 190:1250–1263
35. Fehri LF, Koch M, Belogolova E, Khalil H, Bolz C, Kalali B et al (2010) Helicobacter pylori induces miR-155 in T cells in a cAMP-Foxp3-dependent manner. PLoS One 5:e9500
36. Peres RS, Liew FY, Talbot J, Carregaro V, Oliveira RD, Almeida SL, França RFO, Donate PB, Pinto LG, Ferreira FIS, Costa DL, Demarque DP, Gouvea DR, Lopes NP, Queiroz RHC, Silva JS, Figueiredo F, Alves-Filho JC, Cunha TM, Ferreira SH, Louzada-Junior P, Cunha FQ (2015) Low expression of CD39 on regulatory T cells as a biomarker for resistance to methotrexate therapy in rheumatoid arthritis. Proc Natl Acad Sci 112:2509–2514
37. Boldin MP, Taganov KD, Rao DS, Yang L, Zhao JL, Kalwani M, Garcia-Flores Y, Luong M, Devrekanli A, Xu J, Sun G, Tay J, Linsley PS, Baltimore D (2011) miR-146a is a significant brake on autoimmunity, myeloproliferation, and cancer in mice. J Exp Med 208:1189–1201
38. Liu J, Shi K, Chen M, Xu L, Hong J, Hu B, Yang X, Sun R (2015) Elevated miR-155 expression induces immunosuppression via CD39+ regulatory T-cells in sepsis patient. Int J Infect Dis 40:135–141
39. Kohlhaas S, Garden OA, Scudamore C, Turner M, Okkenhaug K, Vigorito E (2009) Cutting edge: the Foxp3 target miR-155 contributes to the development of regulatory T cells. J Immunol 182:2578–2582
40. Hong H, Yang H, Xia Y (2018) Circulating miR-10a as predictor of therapy response in rheumatoid arthritis patients treated with methotrexate. Curr Pharm Biotechnol 19:79–86
41. Saevarsdottir S, Wedren S, Seddighzadeh M et al (2011) Patients with early rheumatoid arthritis who smoke are less likely to respond to treatment with methotrexate and tumor necrosis factor inhibitors: observations from the Epidemiological Investigation of Rheumatoid Arthritis and the Swedish Rheumatology Register cohorts. Arthritis Rheum 63:26–36
42. Anderson JJ, Wells G, Verhoeven AC, Felson DT (2000) Factors predicting response to treatment in rheumatoid arthritis: the importance of disease duration. Arthritis Rheum 43:22–29
43. O'Dell JR, Nepom BS, Haire C, Gersuk VH, Gaur L, Moore GF, Drymalski W, Palmer W, Eckhoff PJ, Klassen LW, Wees S, Thiele G, Nepom GT (1998) HLA-DRB1 typing in rheumatoid arthritis: predicting response to specific treatments. Ann Rheum Dis 57:209–213
44. Qiu Q, Huang J, Shu X, Fan H, Zhou Y, Xiao C (2017) Polymorphisms and pharmacogenomics for the clinical efficacy of methotrexate in patients with rheumatoid arthritis: a systematic review and meta-analysis. Sci Rep 7:44015
45. Drozdzik M, Rudas T, Pawlik A, Gornik W, Kurzawski M, Herczynska M (2007) Reduced folate carrier-1 80G>A polymorphism affects methotrexate treatment outcome in rheumatoid arthritis. Pharmacogenomics J 7:404–407
46. Nair VS, Pritchard CC, Tewari M, Ioannidis JP (2014) Design and analysis for studying microRNAs in human disease: a primer on -omic technologies. Am J Epidemiol 180:140–152