Serum vitamin D level and micro-ribonucleic acid-146a expression pattern in dry eye disease associated with rheumatoid arthritis in an Egyptian population

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Aim
The aim of this research was to study serum vitamin D and micro-ribonucleic acid-146a (miRNA-146a) expression in dry eye disease (DED) associated with rheumatoid arthritis (RA) and their clinical correlations to DED parameters.
Setting and design
This is an observational cross-sectional study that was conducted at Cairo University, Cairo, Egypt.
Patients and methods
The study participants were divided into three groups: group A, DED/RA (n=35); group B, non-DED/RA (n=36); and group C, non-DED/non-RA (n=35). All participants were assessed for ocular surface disease index, tear breakup time (TBUT), serum level of vitamin D, and miRNA-146a expression. In the DED group (with TBUT<10 s), DED evaluation was performed, including Schirmer I test, corneal and conjunctival scoring, and impression cytology.
Results
The mean serum vitamin D level was 17.1±16.2 ng/ml in group A, 35.1±13.4 ng/ml in group B, and 38.1±8.7 ng/ml in group C, with a statistically significant difference (P<0.001). The mean miRNA-146a expression was 4.7±1.5 in group A compared with 4.8±1.8 in group B (P=0.959) and 1.0±0.1 in group C (P<0.001, compared with each of groups A and B). In group A, serum vitamin D level showed a significant moderate negative correlation to each of impression cytology grading (r=–0.456, P=0.019) and miRNA-146a expression (r=–0.387, P=0.041). Both serum vitamin D and miRNA-146a expression showed nonsignificant correlations to ocular surface disease index, TBUT, Schirmer I test, and corneal and conjunctival scoring.
Conclusion
DED associated with RA showed a statistically significantly lower serum level of vitamin D, which was negatively correlated to impression cytology grading and miRNA-146a expression. Expression of miRNA-146a did not differ between the dry eye and non-dry eye RA groups.

Keywords:
dry eye disease, micro-ribonucleic acid-146a, rheumatoid arthritis, vitamin D

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Introduction

Dry eye disease (DED) is very common in individuals with rheumatoid arthritis (RA), even being the commonest extra-articular manifestation [1]. DED symptoms affect the lifestyle quality. So, continuous research is ongoing about the possible treatment methods and associated risk factors [2]. According to the Tear Film and Ocular Surface Society Dry Eye Workshop (TFOS DEWS II) report in 2017, DED definition was updated to acknowledge the significant role of inflammation and hyperosmolarity within the DED pathway and pathogenesis [3].

Vitamin D has been known to influence the inflammatory process all over the body, modulating many diseases by its anti-inflammatory, anti-oxidant, and immune-modulatory functions [4]. Over the past years, research has been ongoing on the relation of vitamin D to DED and its special correlation to clinical diagnostic parameters of DED in different populations with heterogeneous results [5-10].

Micro-ribonucleic acids (miRNAs) are defined as noncoding, small RNAs, of ~22 nucleotides that act as post-transcriptional regulators of gene expression. They are very resistant to degrading enzymes and are found in all

cells and body fluids. So, they have the potential to serve as biomarkers for several diseases [11,12]. miRNA-146a expression was related to DED in some studies [12-16].

The aim of this work was to study DED in Egyptian patients with RA regarding their serum vitamin D level and miRNA-146a expression with their clinical correlations to DED parameters.

Patients and methods

This is an observational cross-sectional study that was conducted at Cairo University Hospital, Cairo, Egypt. The study protocol was approved by the Faculty of Medicine, Cairo University Research Ethics Committee (N-69-2021). The aim of the study and its possible examination hazards were explained to all study participants. All participants signed a written informed consent to participate in the study and for publication of data before enrollment in the study.

The study included patients with RA, diagnosed according to the 2010 American College of Rheumatology criteria [17], who were referred for ophthalmological assessment.

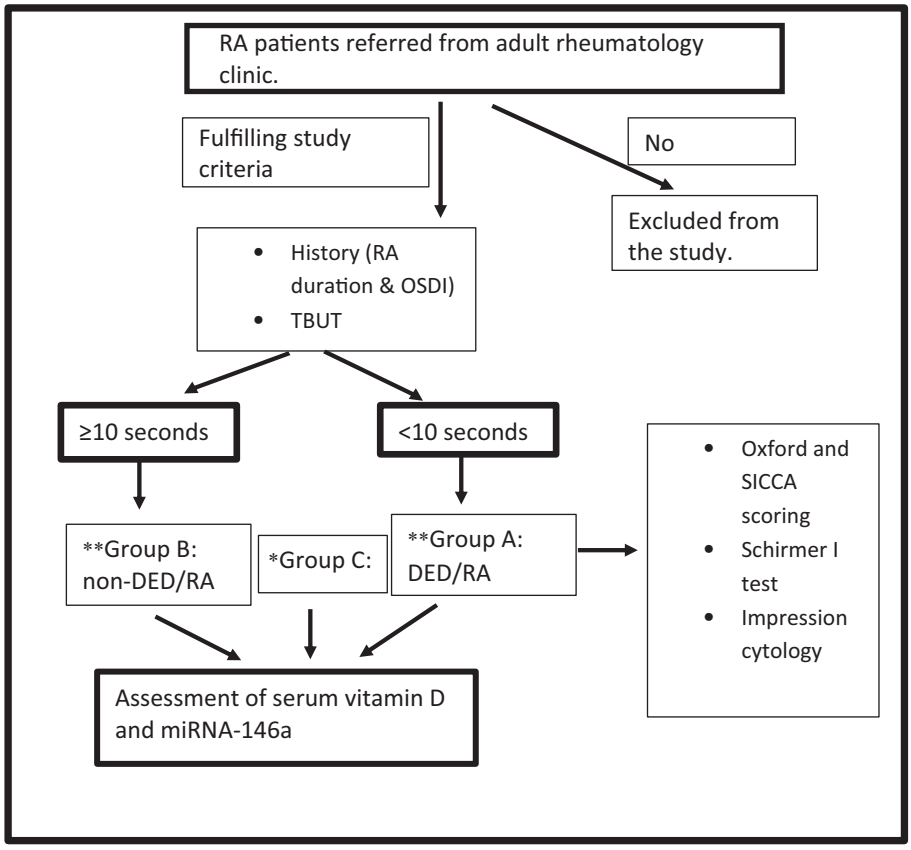
The study participants (n=106) were divided into three groups (that were age and sex matched):

(1) Group A: DED and RA (DED/RA) [based on tear breakup time (TBUT) test <10 s, n=35).
(2) Group B: non-DED and RA (non-DED/RA) (based on TBUT test ≥10 s) (n=36).
(3) Group C: non-DED and non-RA (non-DED/non-RA) (based on TBUT test ≥10 s) (n=35). Group C participants enrolled in the study were patients presenting to the Ophthalmology Clinic diagnosed as non-DED with no history of any associated systemic disease.

Participants on vitamin D treatment and/or lubricant eye drops treatment, cases with history of ocular surgery including refractive or lacrimal surgery or with punctual plugs, cases with chronic use of any eye drops or contact lenses, multiparous women (>3 pregnancies), and postmenopausal women were excluded from the study.

Figure 1 illustrates the assessment and examination flow chart. All study participants were subjected to

Figure 1



Flow chart of the study methodology. DED, dry eye disease; miRNA-146a, micro-ribonucleic acid-146a; OSDI, ocular surface disease index; RA, rheumatoid arthritis; SICCA, Sjogren's International Collaborative Clinical Alliance ocular staining score form; TBUT, tear breakup time. *A third group (non-DED/non-RA) was added. All participants in this group had been assessed for serum vitamin D and miRNA146-a expression in addition to dry eye disease assessment by OSDI and TBUT. **Groups A and B were assessed for RA disease activity scoring.

relevant history taking for RA duration, routine ophthalmological examination, ocular surface disease index (OSDI) questionnaire [18,19], TBUT, assessment of serum level of vitamin D, and miRNA-146a expression.

Dry eye disease parameters

The total score of OSDI was calculated on the basis of the following formula: OSDI=[(sum of scores for all questions answered)×100]/[(total number of questions answered)×4] [18,19].

The TBUT was measured using sterile fluorescein strips, instilled in the inferior fornix. Then, the time taken for the appearance of the first dark spot was recorded, using cobalt blue filtered light of the slit-lamp biomicroscope (TOPCON, Itabashi-ku, Tokyo, Japan). Measurements were repeated three times, and the mean TBUT was calculated [18]. We classified patients into DED when the TBUT was less than 10 s and into non-DED when the TBUT was more than or equal to 10 s [20]. The cases that were classified as DED based on the TBUT were subjected to dry eye evaluation by Schirmer I test, fluorescein and Lissamine green staining for corneal and conjunctival scoring by Oxford and Sjogren's International Collaborative Clinical Alliance ocular staining score form (SICCA) scoring, and impression cytology. Corneal fluorescein staining pattern was observed 2 min following the TBUT test, giving an Oxford staining pattern score to each eye according to Bron *et al.* [21]. In addition, the punctate areas of staining, presence of central fluorescein staining, and/or presence of filaments were noted for the SICCA scoring [22] that was given to each eye. Fluorescein was washed out by a drop of non-preserved saline. Then, one drop of Lissamine green dye 1% (Ophtecnics Unlimited, Trade India, Vasant Vihar, New Delhi, India) was instilled in each eye. The conjunctival staining pattern at the nasal and temporal bulbar conjunctiva was noted by slit-lamp examination according to the SICCA scoring system [22]. After 15 min, Schirmer I test (without anesthesia) was performed using a standardized strip placed in the lateral lower conjunctival sac, to record the amount of wetting after 5 min with the examined eye being closed. For statistical analysis, we considered the mean value of both eyes for each DED parameter.

Serum vitamin D measurement

Serum vitamin D, in the study samples, was quantitatively measured by the competitive immunoassay technique (EIA) using a commercial EIA kit [Epitope Diagnostics Inc. (EDI), San Diego, California, USA, catalog number #Kit (KT)-715]. In this study, serum vitamin D was considered as follows: deficient less than 20 ng/ml, insufficient 20–30 ng/ml, and optimal 30–70 ng/ml [23,24].

Measurement of micro-ribonucleic acid-146a fold change (expression)

(1) Total RNA with preserved micro-RNAs was extracted from 200 µl of serum by a miRNeasy extraction kit (Qiagen, Valencia, California, USA). Then, RNA quantitation and purity assessment using the NanoDrop (ND)-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, Delaware, USA) was done.
(2) Using the miScript II RT kit (Qiagen), the extracted RNA (1 µg) was reverse transcribed to complementary deoxyribonucleic acid in a final volume of 20-µl RT reactions (incubated for 60 min at 37°C, followed by 5 min at 95°C). Real-time quantitative PCR was done using miScript SYBR green PCR kit (Qiagen) that contained miRNA-146a-specific forward primer (5′-CAG-CTG-CAT-TGG-ATT-TAC-CA-3′) and its reverse primer (5′-GCC-TGA-GAC-TCT-GCC-TTC-TG-3′) in addition to U6 as an internal reference (forward primer; 5′-GCTTCGGCAGCACATATACTAAAAT-3′ and reverse primer; 5′-CGCTTCACGAA TTTGCGTGTCAT-3′). After the PCR cycles, melting curve analyses were done to affirm the specific generation of the expected PCR product. The threshold cycle [25] ($2^{-\Delta\Delta Ct}$) method of comparative PCR was used to interpret the results.

Impression cytology

Under topical anesthesia using benoxinate hydrochloride 0.4% (Benox eye drops, EIPICO, Tenth of Ramadan City, Egypt), and using blunt forceps, a filter paper strip (fashioned into an 8-mm strip, with one wide end and one pointing end) was applied gently to the temporal and nasal bulbar conjunctiva. After 10 s, the filter paper was removed to be applied on a slide, fixed with ethyl alcohol, stained with periodic acid-Schiff, counter-stained with hematoxylin, and then examined under a light microscope for the cytological features of the epithelial and goblet cells. A grade was given from one to three according to Nelson *et al.* [26].

Rheumatoid arthritis disease activity

It was assessed in all patients with RA using the clinical disease activity index [27].

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS 26 for Windows) (SPSS Inc., Chicago, Illinois, USA). Quantitative variables were described as mean±SD and were compared using independent *t* test and Mann–Whitney *U* test for two independent groups and Kruskal–Wallis test for three independent groups. Spearman correlation (*r*) was done for quantitative variables (0–0.25=weak or no correlation, 0.25–0.75=moderate correlation, and 0.75–1=strong correlation). Qualitative variables were described as frequency and percentage and were compared using the χ^2 test and Fisher's exact test. A *P* value less than 0.05 was considered statistically significant.

Results

The three study groups were age and sex matched with a nonsignificant difference in smoking history between them ($P>0.05$). The TBUT and OSDI were significantly different in the DED group compared with the other two non-DED groups ($P<0.001$). However, they were not statistically significantly different between the two non-DED groups ($P=0.077$ and 0.058, respectively) (Table 1). The average RA disease duration was 24±21 months in group A and 38±30 months in group B, with a statistically nonsignificant difference ($P=0.071$). The average RA disease activity was 29±14 in group A and 24±10 in group B ($P=0.038$).

Vitamin D level was statistically significantly lower in group A (DED/RA) than both non-DED groups (group B or C, $P<0.001$). There was a statistically nonsignificant difference in vitamin D level between the two non-DED groups (groups B and C, $P=0.0862$). The proportion of low vitamin D (<30 ng/ml) was significantly higher in the DED/RA group (88.6%) when compared with the non-DED/RA (27.7%) and the non-DED/non-RA groups (17.1%) ($P<0.001$) (Table 2). So, low vitamin D was related to DED (Table 2 and Fig. 2).

There was a statistically nonsignificant difference in miRNA-146a expression between the two RA groups (groups A and B) ($P=0.959$), but there was a statistically significant difference between each of the RA groups compared with the non-DED/non-RA group (*P* value between groups A and C ≤0.001, and *P* value between groups B and C≤0.001, Table 2). Thus, high miRNA-146a expression was related to RA rather than to DED (Table 2 and Fig. 3). Table 3 illustrates the DED evaluation parameters in group A patients, which was not applied to the other non-DED groups as mentioned in the examination scheme (Fig. 1).

On further group A analysis, for the correlations between vitamin D level and DED evaluation parameters and RA activity, only a significant moderate negative correlation was found to impression cytology grading ($r=-0.456$, $P=0.019$, Table 4), whereas miRNA-146a expression did not show any significant correlations to DED parameters nor to RA activity (Table 5). Furthermore, vitamin D level and miRNA-146a expression showed a significant moderate negative correlation ($r=-0.387$, $P=0.041$, Table 4).

Table 1 Demographic and dry eye disease evaluation data

Data	Group A (*N*=35 eyes)	Group B (*N*=36 eyes)	Group C (*N*=35 eyes)	*P*
Mean age (years)	37±6	35±6	34±5	0.058
Females (n(%))	31 (88.6)	31(86.1)	31 (88.6)	0.935
Smokers (n(%))	1 (2.9)	2 (5.5)	1 (2.9)	0.990
Average OSDI; score	57.7±31.6	0.0±0.0	4.3±8.6	<0.001*
Average TBUT (s)	5.5±2.0	20.2±6.3	15.7±3.7	<0.001†

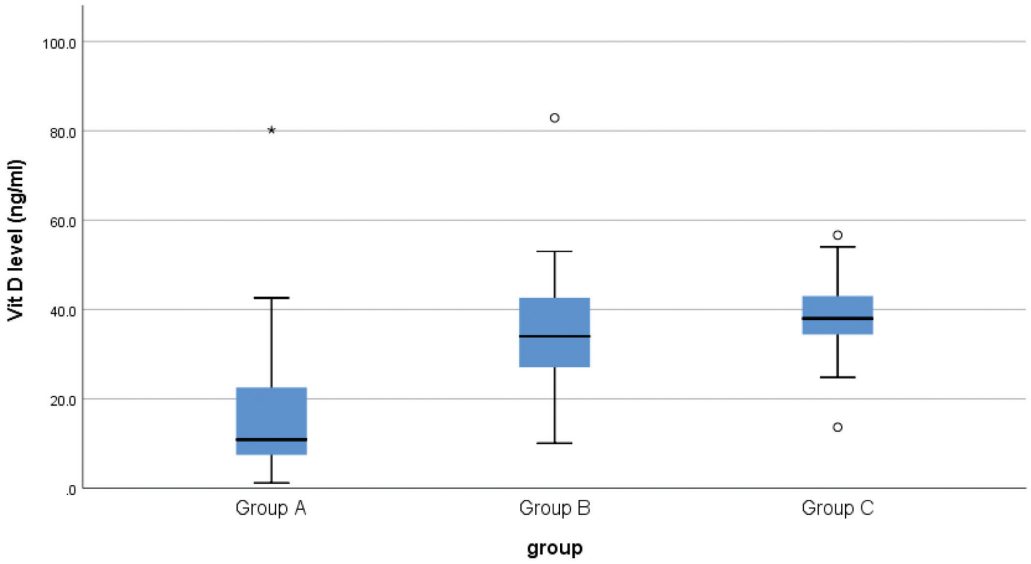
OSDI, ocular surface disease index; TBUT, tear breakup time.
*P value between group A and each of B and C (statistically significant), *P* value between groups B and C=0.0582 (nonsignificant).
†P value between group A and each of B and C (statistically significant), *P* value between groups B and C=0.0768 (nonsignificant).

Table 2 Vitamin D level and micro-ribonucleic acid-146a expression of the three study groups

Variables	Group A (*N*=35 eyes)	Group B (*N*=36 eyes)	Group C (*N*=35 eyes)	*P*
Serum vitamin D (ng/ml)	17.1±16.2	35.1±13.4	38.1±8.7	<0.001‡
Proportion of low vitamin D (<30 ng/ml) (n(%))	31 (88.6)	10 (27.7)	6 (17.1)	<0.001‡
miRNA-146a expression	4.7±1.5	4.8±1.8	1.0±0.1	0.959§

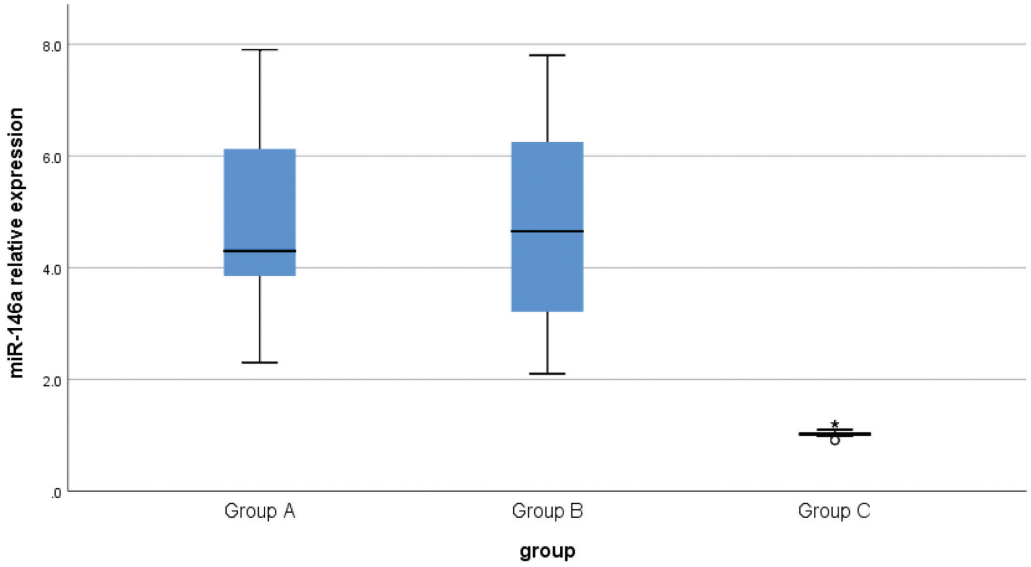
miRNA-146a: micro-ribonucleic acid-146a.
‡*P* value between group A and each of B and C (statistically significant), *P* value between groups B and C=0.0862 (nonsignificant).
§*P* value between groups A and B (nonsignificant), *P* value between groups A and C less than or equal to 0.001, *P* value between group B and C less than or equal to 0.001

Figure 2



Vitamin D level in the 3 study groups. Group A: patients with dry eye disease/rheumatoid arthritis, group B: patients with non-dry eye disease/rheumatoid arthritis, and group C: patients with non-dry eye disease/non-rheumatoid arthritis.

Figure 3



Micro-ribonucleic acid-146a relative expression in the three study groups. Group A: patients with dry eye disease/rheumatoid arthritis, group B: patients with non-dry eye disease/rheumatoid arthritis, and group C: patients with non-dry eye disease/non-rheumatoid arthritis. miR-146a, micro-ribonucleic acid-146a.

Discussion

Recent research has been ongoing on disease biomarkers aiming at easier, earlier prediction, and therapeutic potential of important diseases. DED is one of those diseases. To the best of our knowledge, no previous studies have analyzed both serum vitamin D and miRNA-146a expression in DED associated with RA.

In the current study, there was a statistically significant vitamin D deficiency (17.1±16.2 ng/ml) in the DED/RA group compared with the non-DED/RA group (35.1±13.4 ng/ml) and the non-DED/non-RA group (38.1±8.7 ng/ml), with a nonsignificant difference in serum vitamin D level between the two non-DED groups, denoting that low serum vitamin D was linked to DED. In addition, low vitamin D level in DED was supported as well by the significantly higher proportion

Table 3 Dry eye disease evaluation data in the dry eye disease patients (group A)

Variables	Group A (*N*=35 eyes)
Average Schirmer I (mm/5 min)	8.9±5.9
Average Oxford scoring	4.5±3.1
Average SICCA grading	6.5±3.3
Average impression cytology; grade	1.8±0.7

SICCA, Sjogren's International Collaborative Clinical Alliance ocular staining score form.

Table 4 Correlations of serum vitamin D level to dry eye disease evaluation parameters, rheumatoid arthritis activity, and micro-ribonucleic acid-146a expression in group A (patients with dry eye disease)

Variable 1	Variable 2	Correlation coefficient	Sig. (2-tailed)
OSDI; score	Serum vitamin D; ng/ml	−0.140	0.496
TBUT; (a)	Serum vitamin D; ng/ml	0.066	0.749
Schirmer I test; mm/5 min	Serum vitamin D; ng/ml	0.062	0.764
SICCA; score	Serum vitamin D; ng/ml	−0.337	0.093
Oxford; score	Serum vitamin D; ng/ml	−0.174	0.395
Impression cytology; grade	Serum vitamin D; ng/ml	−0.456	0.019*
miRNA-146a expression	Serum vitamin D; ng/ml	−0.387	0.041*
Disease activity; score	Serum vitamin D, ng/ml	−0.188	0.134

OSDI, ocular surface disease index; SICCA, Sjogren's International Collaborative Clinical Alliance ocular staining score form; miRNA-146a, micro-ribonucleic acid-146a; TBUT, tear breakup time.
*Statistically significant.

Table 5 Correlations of micro-ribonucleic acid-146a expression to dry eye disease evaluation parameters and rheumatoid arthritis activity in group A (patients with dry eye disease)

Variable 1	Variable 2	Correlation coefficient	Sig. (2-tailed)
OSDI; score	miRNA-146a expression	−0.088	0.668
TBUT (s)	miRNA-146a expression	0.070	0.735
Schirmer I test; mm/5 min	miRNA-146a expression	0.062	0.765
SICCA; score	miRNA-146a expression	−0.292	0.147
Oxford; score	miRNA-146a expression	−0.306	0.128
Impression cytology; grade	miRNA-146a expression	−0.141	0.491
Disease activity; score	miRNA-146a expression	−0.056	0.751

miRNA-146a, micro-ribonucleic acid-146a; OSDI, ocular surface disease index; SICCA, Sjogren's International Collaborative Clinical Alliance ocular staining score form; TBUT, tear breakup time.

of low vitamin D (<30 ng/ml) in the DED/RA group (88.6% of the studied group participants) compared with the other two non-DED groups (27.7 and 17.1%, respectively). Vitamin D level showed only a significant negative correlation to impression cytology among the DED evaluation parameters (OSDI, TBUT, Schirmer I test, corneal and conjunctival staining, and impression cytology). We excluded all possible cases with vitamin D deficiency like multiparous and postmenopausal females and any case with a known deficiency.

Several studies reported the association between low vitamin D and DED. A retrospective study by Jin *et al.* [10] investigated the correlation between serum vitamin D and DED parameters in patients with DED. They found a positive correlation to TBUT, with no significant correlation between vitamin D and each of OSDI and fluorescein staining score, like the current study results. They reported deficient vitamin D (14.41±5.98 ng/ml) in patients with DED [10]. Low serum vitamin D in DED was also found by Meng *et al.* [7], but unlike the current results, they found significant correlations between vitamin D level and TBUT, Schirmer test, and OSDI [7]. The study by Yang *et al.* [5] showed that oral vitamin D treatment improved the DED symptoms in an elderly population study group, but unlike the current results, they found a significant correlation to corneal staining scoring system [5]. Another study conducted also on oral vitamin D supplement by Bae *et al.* [28] showed that it was effective in refractory cases of DED [28]. A systematic review and meta-analysis in 2020 by Askari *et al.* [2] concluded that serum vitamin D had a significantly lower level in DED and correlated with OSDI but not to other DED parameters [2]. A recent study by Lee *et al.* [24] showed that low vitamin D was associated with DED in primary Sjogren's syndrome, with significant correlations to TBUT, Schirmer test, and corneal and conjunctival staining but not to OSDI [24]. Two cross-sectional studies [8,29] conducted on a sample of Korean population (in which RA patients were excluded) showed that vitamin D deficiency was associated with DED, but this association was insignificant in multivariate adjusted analysis in the study by Kim *et al.* [8] However, another study by Jee *et al.* [30], which was conducted also on a Korean population, did not support the association between

DED and vitamin D; of note, they based DED diagnosis only on history of a clinical diagnosis of DED by a physician, and this could explain their findings, which are contrary to the current study results as well as to many previously mentioned studies, as they probably missed a lot of DED cases [30]. In addition, in contrary to our results, Jeon *et al.* [9] found a nonsignificant association between vitamin D level and DED. However, DED was diagnosed in their study by OSDI only. So, they probably missed a lot of DED cases [9]. Thus, these studies together with the current study showed that low vitamin D could be highly linked to DED but with very variable correlations to DED parameters. In the present study, there was a negative correlation to impression cytology, and this could be related to the inflammatory link. This inflammation could result in epithelial surface cell changes up to death [2,3,31]. Vitamin D can inhibit interleukin-6 which is an inflammatory modulator. So, vitamin D deficiency can promote more inflammation. Vitamin D improves the epithelial barrier function, which could explain the negative correlation between vitamin D level and impression cytology grading [2,5,32].

In the present study, there was no significant difference in miRNA-146a expression between the DED/RA group and the non-DED/RA group, but there was a significant difference between each of them and the non-DED/non-RA group. This could point that miRNA overexpression could be a marker for RA rather than DED in our population. However, there was a significant negative correlation between vitamin D level and miRNA-146a expression in patients with DED/RA. This might indicate that miRNA-146a expression could be increased in DED/RA if a larger population had been assessed or this correlation could be linked to the discovered anti-inflammatory role of vitamin D through decreasing miRNAs expression, among them miRNA-146a [33].

Contrary to the current study, miRNA-146a overexpression was noted in patients with primary Sjogren's syndrome in some studies, reflecting its anti-inflammatory and immune-modulatory function through affecting several cytokines and inhibiting the messenger RNA. In addition, its overexpression was correlated to DED symptoms in one of these studies [12-15]. In a study by Yin *et al.* [16], it was shown that miRNA-146a-5p could control the inflammatory response in DED through affecting interleukin-1 receptor-associated kinase 1, which is a serine-threonine kinase that mediates Toll-like receptors and interleukin-1 signaling pathways. These signaling pathways regulate the inflammatory processes [16].

Supporting our postulation that miRNA-146a is linked to RA rather than to DED in our population is the increased expression of miRNA-146a, which was found in a population of Egyptian patients with RA by Elsayed *et al.* [34]. However, they did not consider if there was an association to DED or not.

Conclusion

Vitamin D deficiency was linked to DED in patients with RA and was significantly correlated to impression cytology, which suggests its role in epithelial integrity, making vitamin D supplement a potential therapy in these patients. There was no significant change in miRNA-146a expression in DED compared with those classified as non-DED among the patients with RA. However, the current study was carried out on a relatively small sample. So, a larger population-based study is recommended.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Cimmino MA, Salvarani C, Macchioni P, Montecucco C, Fossaluza V, Mascia MT, *et al.* Extra-articular manifestations in 587 Italian patients with rheumatoid arthritis. Rheumatol Int 2000; 19:213–217.

2 Askari G, Rafie N, Miraghajani M, Heidari Z, Arab A. Contact lens and anterior eye association between vitamin D and dry eye disease : a systematic review and meta-analysis of observational studies. Cont Lens Anterior Eye 2020; 43:418–425.

3 Craig JP, Nelson JD, Azar DT, Belmonte C, Anthony JB, Sunil KC, *et al.* TFOS DEWS II report executive summary. Ocul Surf 2017; 15:802–812.

4 Nagpal S, Na S, Rathnachalam R. Noncalcemic actions of vitamin D receptor ligands. Endocr Rev 2005; 26:662–687.

5 Yang CH, Albietz J, Harkin DG, Kimlin MG, Schmid KL. Impact of oral vitamin D supplementation on the ocular surface in people with dry eye and/or low serum vitamin D. Cont Lens Anterior Eye 2018; 41:69–76.

6 Shetty R, Sethu S, Deshmukh R, Deshpande K, Ghosh A, Agrawal A, Shroff R. Corneal dendritic cell density is associated with subbasal nerve plexus features, ocular surface disease index, and serum vitamin D in evaporative dry eye disease. Biomed Res Int 2016; 2016:4369750.

7 Meng YF, Lu J, Xing Q, Tao JJ, Xiao P. Lower serum vitamin D level was associated with risk of dry eye syndrome. Med Sci Monit 2017; 23:2211–2216.

8 Kim MJ, Hwang HR, Kim Y-J, Lee S-Y, Lee J-G, Jeong D-W, Kim YH. Association between serum 25-hydroxyvitamin D levels and dry eye in Korean adults: a study based on Korean National Health and Nutrition Examination Survey, 2010–2011. Korean J Fam Med 2017; 38:81.

9 Jeon D-H, Yeom H, Yang J, Song JS, Lee HK, Kim HC. Are serum vitamin D levels associated with dry eye disease? Results from the study group for environmental eye disease. J Prev Med Public Health 2017; 50:369–376.

10 Jin KW, Ro JW, Shin YJ, Hyon JY, Wee WR, Park SG. Correlation of vitamin D levels with tear film stability and secretion in patients with dry eye syndrome. Acta Ophthalmol 2017; 95:e230–e235.

11 Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet 2010; 11:597–610.

12 Meyre D, Paiva CS, Cristina L, Modulo CM, Adriano L, Fantucci MZ, Rocha EM. The ocular surface review : microRNAs in ocular surface and dry eye diseases. Ocul Surf 2017; 15:660–669.

13 Shi H, Zheng L, Zhang P, Yu C. miR-146a and miR-155 expression in PBMCs from patients with Sjögren's syndrome. J Oral Pathol Med 2014; 43:792–797.

14 Pauley KM, Stewart CM, Gauna AE, Dupre LC, Kuklani R, Chan AL, *et al.* Altered miR-146a expression in Sjögren's syndrome and its functional role in innate immunity. Eur J Immunol 2011; 41:2029–2039.

15 Reale M, Angelo CD, Costantini E, Laus M, Moretti A, Croce A. Review article microRNA in Sjögren's syndrome : their potential roles in pathogenesis and diagnosis. J Immunol Res 2018; 2018:7510174.

16 Yin L, Zhang M, He T, Chen S. The expression of miRNA-146a-5p and its mechanism of treating dry eye syndrome. J Clin Lab Anal 2021; 35:e23571.

17 Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham IIICO, *et al.* Rheumatoid arthritis classification criteria : an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Ann Rheum Dis 2010; 69:1580–1588.

18 Ozcura F, Aydin S, Helvaci MR. Ocular surface disease index for the diagnosis of dry eye syndrome. Ocul Immunol Inflamm 2007; 15:389–393.

19 Schiffman RM, Dale CM, Jacobsen G, Hirsch JD, Reis BL. Reliability and validity of the ocular surface disease index. Arch Ophthalmol 2000; 118:615–621.

20 Tutt R, Bradley A, Begley C, Thibos LN. Optical and visual impact of tear break-up in human eyes. Invest Ophthalmol Vis Sci 2000; 41:4117–4123.

21 Bron AJ, Evans VE, Smith JA. Grading of corneal and conjunctival staining in the context of other dry eye tests. Cornea 2003; 22:640–650.

22 Whitcher JP, Shiboski CH, Shiboski SC, Heidenreich AM, Kitagawa K, Zhang S, *et al.* A simplified quantitative method for assessing keratoconjunctivitis sicca from the Sjögren's syndrome international registry. Am J Ophthalmol 2010; 149:405–415.

23 Adami S, Romagnoli E, Carnevale V, Scillitani A, Giusti A, Rossini M, *et al.* Guidelines on prevention and treatment of vitamin D deficiency. Italian Society for Osteoporosis, Mineral Metabolism and Bone Diseases. Reumatismo 2011; 63:129–147.

24 Lee JH, Kim SJ, Byun Y, Lee J. The association of serum vitamin D level with the severity of dry eye parameters in primary Sjogren syndrome. Cornea 2020; 39:702–705.

25 Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative CT method. Nat Protoc 2008; 3:1101–1108.

26 Nelson JD, Havener VR, Cameron JD. Cellulose acetate impressions of the ocular surface: dry eye states. Arch Ophthalmol 1983; 101:1869–1872.

27 Aletaha D, Nell VPK, Stamm T, Uffmann M, Pflugbeil S, Machold K, Smolen JS. Acute phase reactants add little to composite disease activity indices for rheumatoid arthritis: validation of a clinical activity score. Arthr Res Ther 2005; 7:796–806.

28 Bae SH, Shin YJ, Kim HK, Hyon JY, Wee WR. Vitamin D supplementation for patients with dry eye syndrome refractory to conventional treatment. Nature Publishing Group scientific report. Sci Rep 2016; 6:33083.

29 Yoon SY, Bae SH, Shin YJ, Park SG, Hwang S-H, Hyon JY, Wee WR. Low serum 25- hydroxyvitamin D levels are associated with dry eye syndrome. PLoS ONE 2016; 11:e0147847.

30 Jee D, Kang S, Yuan C, Cho E, Arroyo JG. The Epidemiologic Survey Committee of the Korean Ophthalmologic Society (2016) serum 25-hydroxyvitamin D levels and dry eye syndrome: differential effects of vitamin D on ocular diseases. PLoS ONE 2016; 11:e0149294.

31 Baudouin C, Aragona P, Messmer EM, Tomlinson A, Calonge M, Boboridis KG, *et al.* Role of hyperosmolarity in the pathogenesis and management of dry eye disease: proceedings of the ocean group meeting. Ocul Surf 2013; 11:246–258.

32 Yin Z, Pintea V, Lin Y, Hammock BD, Watsky MA. Vitamin D enhances corneal epithelial barrier function. Investig Ophthalmol Vis Sci 2011; 52:7359–7364.

33 Karkeni E, Bonnet L, Marcotorchino J, Tourniaire F, Astier J, Ye J, Landrier JF. Vitamin D limits inflammation-linked microRNA expression in adipocytes in vitro and in vivo: a new mechanism for the regulation of inflammation by vitamin D. Epigenetics 2018; 13:156–162.

34 Elsayed HMA, Khater WS, Ibrahim AA, Hamdy MSE, Morshedy NA. MicroRNA-146a expression as a potential biomarker for rheumatoid arthritis in Egypt. Egypt J Med Hum Genet 2017; 18:173–179.