Original Research Article

The Egyptian Journal of Immunology

The Egyptian Journal of Immunology,
E-ISSN (2090-2506)
Volume 31 (3), July, 2024
Pages: 131–139.
www.Ejimmunology.org
https://doi.org/10.55133/eji.310313

MicroRNA-155 is a potential predictive tool for atopic dermatitis severity in children: A preliminary study

Lobna A. El-Korashi[1], Ola E. Nafea[2], Alaa E. Nafea[3], Basma M. Elkholy[4], Lamia L. Elhawy[5], and Amina A. Abdelhadi[1]

[1]Department of Medical Microbiology & Immunology, Faculty of Medicine, Zagazig, University, Zagazig, Egypt.

[2]Forensic Medicine & Clinical Toxicology Department, Faculty of Medicine, Zagazig, University, Zagazig, Egypt.

[3]Department of Pediatrics, Faculty of Medicine, Zagazig University, Zagazig, Egypt.

[4]Department of Dermatology, Venereology & Andrology, Faculty of Medicine, Zagazig University, Zagazig, Egypt.

[5]Department of Community Medicine, Faculty of Medicine, Zagazig University, Zagazig, Egypt.

Corresponding author: Lobna A. El-Korashi, Department of Medical Microbiology & Immunology, Faculty of Medicine, Zagazig, University, Zagazig, Egypt.
Email: alqurashilobna@yahoo.com

Abstract

Atopic dermatitis (AD) is one of the most prevalent chronic inflammatory dermatological disorders in childhood. Assessment of AD severity is the initial step in designing the proper therapeutic plan. Moreover, it is imperative for evaluation of disease improvement during and following therapy. This study was designed to assess the prognostic role of miRNA-155 (miR-155) in the prediction of AD severity as the primary outcome. While the secondary outcome was to correlate the serum miR-155 expression levels with the scoring atopic dermatitis (SCORAD) severity index. This case-control study included 24 children with AD and 24 apparently healthy children as a control group. AD children were stratified according to the SCORAD severity index. Approximately 58% of children had mild AD, 25% moderate AD, and about 17% severe AD. Children with AD had statistically significantly higher miR-155 expression levels in comparison to the control children, (p< 0.001). Children with severe AD had statistically significantly higher miR-155 expression levels compared to mild AD children (p=0.001). The receiver operating characteristic curve analysis for miR-155 demonstrated that miR-155 can differentiate between children with mild AD and those with moderate-to-severe AD, with an area under the curve of 0.879, and an excellent discrimination power. A statistically strong significant positive correlation existed between miR-155 levels and SCORAD severity index (r_s= 0.666, p<0.001). In conclusion, MiR-155 could be considered as a non-invasive biomarker of AD severity in children. It is a promising prognostic tool in the prediction of AD severity.

Keywords: AD, MicroRNA-155, Predication, ROC curve analysis, SCORAD, Severity

Date received: 20 January 2024; **accepted:** 29 June 2024

Introduction

Atopic dermatitis (AD) is one of the most prevalent chronic inflammatory dermatological disorders that usually starts in childhood but often continues to adulthood, putting a major burden on healthcare costs. AD is manifested by exaggerated eczema and itching negatively

influencing the quality of life of both children and their family members. AD is the most common non-fatal health concern related to dermatological disorders which has a considerable psychosocial impact on patients and their families.[1-5]

The reported global prevalence of AD is 15-20% in pediatric population and 1-3% in adults.[6] Albeit the prevalence of AD and its burden varies extensively across the world. The estimated prevalence range of AD among Egyptian children has been reported as 3.6–12.01%.[7,8] Environmental factors, ethnicity, and socioeconomic status are the key determinants of its prevalence.[9] AD is a highly heritable disease. It usually appears with other atopic conditions including bronchial asthma, allergic rhinitis, and food allergies.[6,10]

The underlying pathophysiology of AD is multifactorial. Structural protein filaggrin insufficiency, disturbance of epidermal barrier function, and stimulation of the inflammatory processes and T cell infiltration are the commonly proposed AD mechanisms. In addition to other associated factors including, *Staphylococcus aureus* invasion, systemic immune responses [immunoglobulin E (IgE)-mediated sensitization] as well as neuroinflammation.[11,12]

The diagnosis of AD is mainly clinical. The American Academy of Dermatology (AAD) has developed simple diagnostic criteria based on symptoms and signs of the affected patients.[13]

Assessment of AD severity is the initial step in designing the proper therapeutic plan for the patient. Moreover, it is imperative for evaluation of disease improvement during and following therapy.[14,15] Various scoring systems have been developed for the assessment of AD severity. SCORing Atopic Dermatitis (SCORAD) considers clinical findings and area of involvement along with a subjective assessment of pruritus and sleep. Although SCORAD is a valid tool in AD severity assessment, it is time consuming during routine clinical examination and subjected to a high risk of biased self-reported data.[14, 16, 17]

MicroRNAs (miRNAs) are a class of a short non-coding single-stranded RNA molecules, attached to the 3’ untranslated region of target messenger RNAs (mRNAs) and control the process of translation.[18] MiRNAs have gained scientific significance because of their involvement in the pathophysiology of allergic disorders and their promising role as biomarkers in liquid biopsies.[19] MiR-155 has been incriminated in many inflammatory immunological dermatological diseases.[20]

MiR-155 has been involved in IgE-dependent allergic disorders comprising AD and asthma.[21] MiR-155 levels were markedly overexpressed in different biological samples in patients with various forms of allergic conditions. For example, high expression levels of miR-155 were recognized in serum samples of patients with allergic rhinitis, in asthmatic air passages and plasma.[20, 22, 23]

Individuals suffering from AD exhibit a marked overexpression of miR-155.[24] Activation of T cells within the skin is a common pathological finding in AD. MiR-155 may have a role in persistent skin inflammation by augmenting the proliferative response of T-helper cells through the downregulation of cytotoxic T lymphocyte–associated antigen 4, a negative regulator of T-cell activation.[25]

This study was designed to evaluate the prognostic role of miRNA-155 in the prediction of AD severity as the primary outcome. While the secondary outcome was to correlate the serum miRNA-155 expression levels with the SCORAD severity index.

Subjects and Methods

This was a case-control study. A total of 24 children with AD were selected to participate from those attending the Allergy and Immunology Unit, Department of Medical Microbiology and Immunology, the Dermatology, Pediatrics Outpatient Clinics, Zagazig University Hospitals, Zagazig, Egypt, between December 2020 to June 2021. In addition, 24 of apparently healthy children after 1:1 matching on age and sex were involved in the study as the control group.

The eligibility criteria included children with AD, diagnosed according to Rajka criteria,[26] listed in the guidelines of the American Academy of Dermatology,[13] and the degree of

severity was assessed by the SCORing Atopic Dermatitis (SCORAD) index (mild<25, moderate=25-50, severe>50).[27]

The exclusion criteria included children who received systemic glucocorticoids, immuno-suppressants or desensitization therapy in the past six months as well as those received local glucocorticoids or calcineurin inhibitors in the previous week. Moreover, children with chronic diseases, rather than asthma, autoimmune disorders, or any associated skin diseases were excluded.

All eligible children were subjected to a full history taking and complete dermatological and clinical examination.

Skin prick test (SPT)

All AD children were checked for skin prick test (SPT). Skin testing was accomplished based on the proposed method of Bernstein et al., 2008. A week prior to skin testing, children were instructed to cease taking antihistamines. Allergen extracts for skin testing were locally prepared at Allergy and Immunology Unit, Department of Medical Microbiology and Immunology, Faculty of Medicine, Zagazig University, Zagazig, Egypt. Home-made allergen extracts: house dust, hay dust, wool, cotton, mixed molds, and mixed pollens were used. Saline and histamine were used as negative and positive controls, respectively. Fifteen minutes after skin prick, the reactions were assessed through measuring the diameters of both wheal and erythema; 3 mm above the negative control was determined as a positive test result.[28]

Peripheral venous blood samples (5 ml) were drawn under complete aseptic conditions from the included children to measure the serum total IgE and expression level of miR-155. Blood samples were centrifuged (1900 × g at 4 °C) for 10 min. Sera were carefully collected and transferred into RNase-free tubes and stored at −80 °C until further analysis of total IgE and miR-155

Serum levels of total IgE

The serum level of total IgE was measured quantitatively by utilizing commercially available sandwich enzyme-linked immunosorbent assay (ELISA) Kits (IMMUNOSPEC Corporation Canoga Park, CA 91303, USA) following the manufacturer's guidance. The results were reported in IU/ml. Using an ELISA reader for microtiter plates (Biotek, USA), the absorbance of standards and samples was measured at 450 nanometers.

Total RNA extraction

RNA extraction was performed using the miRNeasy Serum/Plasma Kits (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The miRNeasy Serum/Plasma Kits combine phenol/guanidine-based lysis of samples and silica-membrane–based purification of total RNA. A spectrophotometer (Thermo Scientific, USA) was used to measure the absorbance at 260 and 280 nm to verify the extracted RNA's quality and purity.

Quantitative real-time polymerase chain reaction (qRT-PCR) for miR-155

miScript II RT Kits (Qiagen, Germany) were utilized to create complementary DNA (cDNA), through reverse transcription process based on the manufacturer's recommendations. The reaction mixture (total volume of 20 µl) was prepared as follows: 2 µl Template RNA, 2 µl 10x miScript Nucleics Mix, 2 µl miScript Reverse Transcriptase Mix, 4 µl 5x miScript HiSpec Buffer, and 10 µl RNase-free water. This mixture was incubated at 37°C for 60 minutes. After that, for inactivation of miScript RT, the mixture was incubated for 5 min at 95°C on a "Rocker Sahara 320 dry bath heat block" and thereafter put promptly onto ice. Afterwards, cDNA was kept at -80 °C until needed.

Serum levels of MiR-155 were quantified by SYBR green-based real-time polymerase chain reaction (qRT-PCR) master mix {mi-Script SYBR® Green PCR Kit with miScript Primer assays (Qiagen, Valencia, CA, USA)}. The amplification was implemented in qRT-PCR (Stratagene Mx3000p system, Agilent Technologies, Germany). The housekeeping small RNA (RNU-6) (miScript PCR control, Qiagen, Germany) was chosen as an internal control based on an earlier research study.[29]

The amplification reaction mixture (20 µl) was prepared in accordance with the kit

manufacturer's instructions as follows: 2 µl of template cDNA, 10 µl 2x QuantiTect SYBR Green PCR Master Mix, 2 µl 10x miScript Primer Assay (miR-155 or RNU-6), 2 µl 10x miScript Universal Primer, and 4 µl RNase-free water. For every sample, two distinct tubes were prepared: one for miR-155 and the other for human RNU6B (RNU6-2) a widely used reference. Thermal cycling condition was as follows: an initial activation step of 15 min at 95°C to activate HotStar Taq DNA Polymerase, followed by 40 cycles each of denaturation at 95°C for 15 sec, then annealing at 55°C for 30 sec, and extension at 70°C for 30 sec.

Normalization of miR-155 expression levels

The ΔCt value and comparative cycle threshold method ($^{\Delta\Delta Ct}$ method) were used to report the miR-155 expression values. The ΔCt value was calculated by subtracting the cycle threshold (Ct) values of miRNA RNU6 from the Ct values of the target miRNAs. Following the demonstration of approximately equal efficiencies between the target gene and reference (miRNA RNU6B) gene in a validation experiment, the comparative threshold method was employed. Using the same sample, standard curves were conducted for each amplicon to determine the relative efficiencies of the target and reference amplifications. In the current study, the $2^{-\Delta\Delta CT}$ method was employed to determine the fold changes of miR- 155 expression.[30]

Statistical Analysis

All statistical analyses were calculated by the GraphPad Prism Software, Version 8.0 (GraphPad, San Diego, CA, United States). Quantitative variables were summarized as the mean± standard deviation (SD) or median (IQR: interquartile range; 25th to 75th percentiles) according to the normality of the data. Qualitative variables were summarized by frequency(percentage). The Shapiro-Wilk test was used to examine the normality of quantitative variables. The receiver operating characteristic (ROC) curve was used to check the prognostic performance of miR-155 in the prediction of AD severity. Spearman's correlation was used to assess the strength and direction of association between two variables, at least one is ordinal. The optimal cutoff points were determined by Youden's index (sensitivity + specificity -1) and the maximized area under the curve (AUC). Differences were considered significant at $p<0.05$. All statistical comparisons were two-tailed.

Results

A total of 24 (6 boys, 18 girls) AD children and 24 (8 boys, 16 girls) normal control children were enrolled in this work. The mean age of the AD children was 7.8± 2.9 years and that of the control children was 7.7±2.6 years. Baseline characteristics were similar between the control children and AD children ($p>0.05$), as presented in Table 1. AD children were stratified according to the SCORAD severity index. Approximately 58% of children had mild AD (14/24), 25% had moderate AD (6/24), and about 17% had severe AD (6/24). Moreover, atopic conditions (allergic conjunctivitis, allergic rhinitis, and asthma), SPT results, and serum levels of total IgE were not different among AD children stratified as stated in the SCORAD index ($p>0.05$), (Table 2).

Table 1. Baseline characteristics of the participating children

Baseline characteristics	Control children	AD children	*p*-value
	n=24	*n*=24	
Age (years), mean± SD	7.8±3.0	7.7±2.6	NS†
Sex, *n* (%)			
Boys	8 (33)	6 (25)	NS‡
Girls	16 (67)	18 (75)	

Data are presented as mean± standard deviation unless otherwise mentioned. $p > 0.05$ is not significant (NS).

†Independent samples t-test. ‡Fisher's exact test (R×C). Abbreviations: AD, atopic dermatitis.

Table 2. Baseline characteristics of atopic dermatitis (AD) children stratified according to SCORAD index.

Baseline characteristics	SCORAD index			*p*-value
	Mild	Moderate	Severe	
	n=14	*n*=6	*n*=4	
Age (years), mean± SD	8.1±3.2	8.7±2.7	5.6±1	NS†
Boys, *n* (%)	6(43)	0(0)	0(0)	NS‡
Associated atopic conditions, *n* (%)				
Allergic conjunctivitis	2(14)	0(0)	0(0)	NS‡
Allergic rhinitis	6(43)	0(0)	0(0)	NS‡
Asthma	6(43)	2(33)	0(0)	NS‡
SPT, *n* (%)				
Mixed molds	4(29)	2(33)	0(0)	NS‡
Mixed pollens	10(71)	4(67)	4(100)	NS‡
House dust	6(43)	2(33)	2(50)	NS‡
Wool	4(29)	0(0)	2(50)	NS‡
Cotton dust	4(29)	0(0)	0(0)	NS‡
Hay dust	2(14)	2(33)	0(0)	NS‡
Total IgE (IU/ml), median (IQR)	42.81(28.83-67.78)	71(22.75-109)	87.5(45-115.75)	NS§

†Independent samples t-test $p > 0.05$ is not significant (NS). ‡Fisher's exact test (R×C). §Kruskal-Wallis *H* test.
Abbreviations: IQR, interquartile range; SCORAD, scoring atopic dermatitis; SPT, skin prick test.

Children with AD had statistically significantly higher miR-155 expression levels compared to the control children [(median (IQR) 16.75 (13.0-23.63) vs. 0.91 (0.25-1.44), ($p< 0.001$)] (Figure 1). Similarly, children with severe AD had statistically significantly higher miR-155 expression levels compared to mild AD children (mean± SD, 26.50±3.46 vs 14.42±6.10, p=0.001). However, miR-155 expression levels did not differ between severe and moderate AD children (mean± SD, 26.50±3.46 vs 20.7±2.90, p=0.21) (Figure 2). Additionally, a statistically significantly positive correlation existed between miR-155 expression levels and SCORAD severity index (r_s= 0.666, p<0.001) (Figure 3). The ROC curve analysis for miR-155 expression level demonstrated that miR-155 can differentiate between children with mild AD and those with moderate-to-severe AD, with an AUC of 0.879, and an excellent discrimination power [(95% confidence interval: 0.725 to 1.0, p=0.002)] (Table 3 and Figure 4).

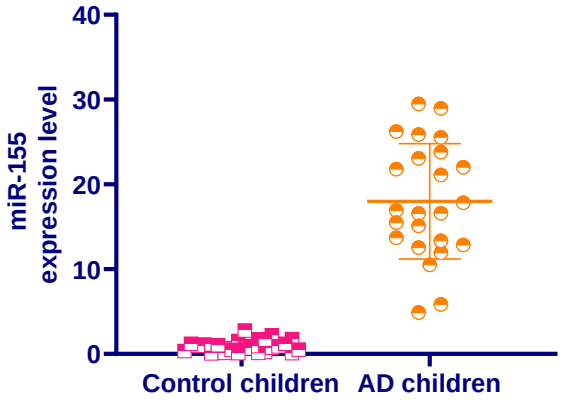

Figure 1. MiR-155 expression levels in the control and atopic dermatitis (AD) children. Data are median (IQR).

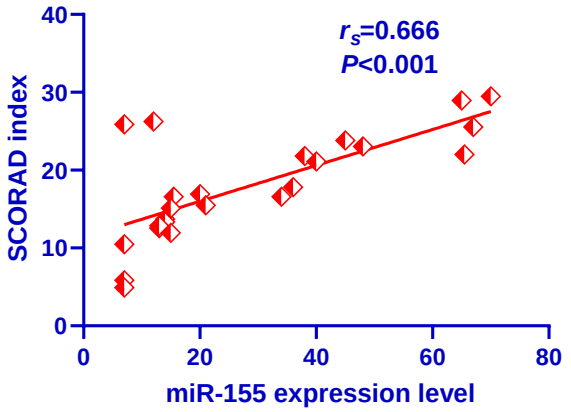

Figure 2. A scatter plot with regression line of miR-155 against SCORAD severity index.
Abbreviations: miRNA, microRNA; rs, Spearman correlation coefficient; SCORAD, scoring atopic dermatitis.

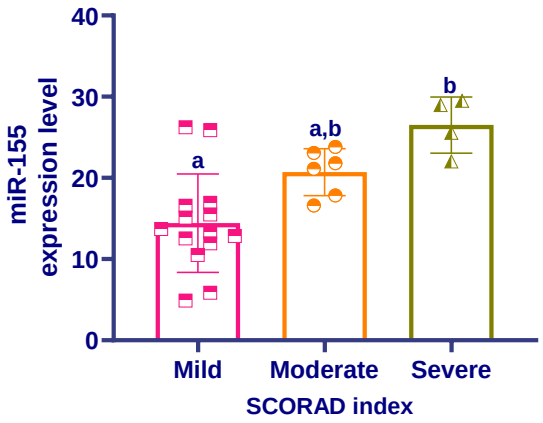

Figure 3. MiR-155 expression levels in atopic dermatitis (AD) children stratified according to the SCORAD severity index. Bars not sharing the common superscript letters differ significantly at $p<0.05$ by ANOVA followed by post hoc Tukey's multiple comparisons test. Data are mean± SD. Abbreviation: miRNA, microRNA; SCORAD, scoring atopic dermatitis.

Table 3. Prognostic value of miR-155 in the prediction of atopic dermatitis (AD) severity.

AD severity	Cut-off value	Sensitivity% (95% CI)	Specificity% (95% CI)	Youden index	C Statistic/AUC (95% CI)	*p*-value
miR-155 expression level	>16.57	100 (72.25-100)	78.6 (52.41-92.43)	0.786	0.879 (0.725 to 1)	0.002

Significant difference at the $p<0.05$ level. Abbreviations: AD, atopic dermatitis; AUC, area under the curve; CI, confidence interval.

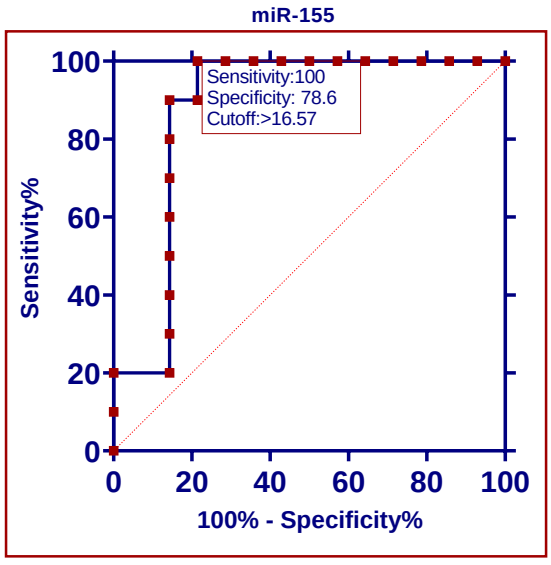

Figure 4. The receiver operating characteristic (ROC) curve for miR-155 in the prediction of atopic dermatitis (AD) severity among the participating AD children.

Discussion

AD is the most frequent chronic inflammatory condition affecting the skin. Inheritance, disrupted skin barrier disruption, and dysregulated immune response are some of the fundamental theories of AD development.[32]

This study was designed to evaluate the prognostic role of miRNA-155 in the prediction of AD severity and to correlate the serum miRNA-155 expression levels with the SCORAD severity index. The results demonstrated that serum miRNA-155 is a promising prognostic biomarker for the assessment of AD severity with an excellent discriminative ability. Moreover, AD severity was positively correlated with serum miRNA-155 expression levels.

MiRNAs act as key players in post-transcription controlling most cellular processes. A single miRNA molecule can target several mRNAs, usually within the same

signaling pathway. Disturbed miRNAs expressions may alter cellular responses and cause disease development. Currently, the role of miRNA in allergy disorders has been extensively studied.[19,33]

MiR-155 plays a master role in innate and adaptive immune responses.[34-36] For example, miRNA-155 is critical for the dendritic cells (DCs) maturation and further activation. Activated DCs are responsible for stimulation of antigen-specific T-cell activation which is one of the main drivers in the pathogenesis of AD.[37, 38]

The present findings showed that the serum expression levels of miRNA were significantly higher in AD children than in control children. In corroboration, the earlier study of Ma and coworkers (2015).[24]demonstrated that the relative expression levels of miRNA-155 in peripheral CD4+ T cells were markedly elevated in 33 AD patients versus 31 healthy participants. Furthermore, they reported that the miRNA-155 relative expression levels in the skin lesions of AD patients were significantly increased in skin lesions compared with perilesional skin and normal skin. According to the study of Sonkoly and colleagues (2010), high expression levels of miR-155 were observed in mast cells from skin lesions of AD patients.[25]

We found a strong positive correlation between miR-155 serum expression levels and SCORAD severity index, this finding reflecting the reported role of miR-155 in the pathogenesis of AD in children. Inconsistent to our findings, Ma et al., 2015, found a moderate positive correlation between miR-155 expression levels in AD peripheral CD4+ T cells with AD disease severity.[24]

AD is associated with the increased risk of immune-mediated inflammatory diseases such as asthma, and allergic rhinitis [5]. In support of this notion, most of the enrolled AD children, especially children with mild AD experience coexistent topic disorders (allergic conjunctivitis, allergic rhinitis, and asthma).

IgE signifies a crucial role in different allergic disorders.[39] However, our findings demonstrated a non-significant difference in serum total IgE levels among AD children stratified according to the SCORAD severity index. Likewise, some people with severe AD may have normal serum total IgE. In addition, possible confounders as coexistent of parasitic infection, and certain types of cancer may alter its level.[40,41]

The present study has some limitations. First, the current findings are considered preliminary until validated in a larger and multicenter study in the future for a more comprehensive understanding the interplay between miRNA-155 and AD, however, the achieved post-hoc power is more than 99%. Second, whether the role of miR-155 in childhood AD differs from adulthood AD is another issue.

In conclusion, MiR-155 serum expression levels were prominently higher in AD children in comparison to apparently healthy control children. MiR-155 could be considered as a non-invasive biomarker of AD severity in children. It is a promising prognostic tool in the prediction of severity AD.

Author Contributions

LAE and AAA conceived the study, study design, participated in biological samples collection, performing the allergic tests, and biochemical analyses. The first draft of the manuscript was written by LAE, AAA, LLE and OEN. OEN performed data analysis and wrote the original draft. AEN, and BME performed the clinical assessment of the enrolled children. All authors participated in drafting, and reviewing of the manuscript, approved the final version, and agreed to be accountable for all aspects of the study.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) denies receipt of any financial support for the research, authorship, and/or publication of this article.

Ethical approval

The study proposal was reviewed and approved by the Institutional Review Board of the Faculty of Medicine, Zagazig University (Approval number, ZU-IRB #6519, November 2020).

Informed consent

An informed written consent was obtained from caregivers of each study child and assent obtained from all study participated children.

References

1. Terzić Z, Mikić M, Ljaljević A, et al. (2023). The long-term efficacy of heliotherapy in ameliorating disease severity and improving the quality of life in patients with atopic dermatitis., Postep. Dermatologii i Alergol. 40 (2023) 159–164. https://doi.org/10.5114/ada.2022.124681.
2. Giavina-Bianchi M, Rizzo L.V, Giavina-Bianchi P (2020). Severe atopic dermatitis: Dupilumab is not just safer, but more efficient, Allergol. Immunopathol. (Madr). 48 (2020) 792–797. https://doi.org/10.1016/j.aller.2019.12.005.
3. Na C.H, Chung J, Simpson E.L (2019). Quality of life and disease impact of atopic dermatitis and psoriasis on children and their families., Child. (Basel, Switzerland). 6 (2019). https://doi.org/10.3390/children6120133.
4. Ständer S (2021). Atopic dermatitis, N. Engl. J. Med. 384 (2021) 1136–1143. https://doi.org/10.1056/NEJMra2023911.
5. Weidinger S, Novak N (2016). Atopic dermatitis, Lancet. 387 (2016) 1109–1122. https://doi.org/10.1016/S0140-6736(15)00149-X.
6. Nutten S (2015). Atopic dermatitis: Global epidemiology and risk factors, Ann. Nutr. Metab. 66 (2015) 8–16. https://doi.org/10.1159/000370220.
7. Maspero J., De Paula Motta Rubini N., Zhang J., et al (2023). Epidemiology of adult patients with atopic dermatitis in AWARE 1: A second international survey. World Allergy Organ J. 2023;16:100724. doi: 10.1016/j.waojou.2022.100724.
8. Al Dhduh M.A.A., Mohamed Sabri N.A., Fouda E.M (2015). Prevalence and Severity of Allergic Diseases among Egyptian Pediatric in Different Egyptian Areas. Int. J. Pharm. Sci. 2015;2:107. doi: 10.15344/2394-1502/2015/107
9. Mosam A, Todd G (2023). Global epidemiology and disparities in atopic dermatitis., Br. J. Dermatol. 188 (2023) 726–737. https://doi.org/10.1093/bjd/ljad042.
10. Løset M, Brown S.J, Saunes M, Hveem K (2019). Genetics of atopic dermatitis: From DNA sequence to clinical relevance, Dermatology. 235 (2019) 355–364. https://doi.org/10.1159/000500402.
11. Weidinger S, Beck L.A, Bieber T, Kabashima K, Irvine A.D (2018). Atopic dermatitis, Nat. Rev. Dis. Prim. 4 (2018) 1. https://doi.org/10.1038/s41572-018-0001-z.
12. Cabanillas B, Novak N (2016). Atopic dermatitis and filaggrin., Curr. Opin. Immunol. 42 (2016) 1–8. https://doi.org/10.1016/j.coi.2016.05.002.
13. Eichenfield L.F, Tom W.L, Chamlin S.L et al. (2014). Guidelines of care for the management of atopic dermatitis: section 1. Diagnosis and assessment of atopic dermatitis., J. Am. Acad. Dermatol. 70 (2014) 338–51. https://doi.org/10.1016/j.jaad.2013.10.010.
14. Fishbein A.B, Silverberg J.I, Wilson E.J, Ong P.Y (2020). Update on atopic dermatitis: Diagnosis, severity assessment, and treatment selection., J. Allergy Clin. Immunol. Pract. 8 (2020) 91–101. https://doi.org/10.1016/j.jaip.2019.06.044.
15. Oranje A.P, Glazenburg E.J, Wolkerstorfer A, d et al. (2007). Practical issues on interpretation of scoring atopic dermatitis: the SCORAD index, objective SCORAD and the three-item severity score, Br. J. Dermatol. 157 (2007) 645–648. https://doi.org/10.1111/j.1365-2133.2007.08112.x.
16. Gooderham M.J, Hong C, Albrecht L et al. (2018). Approach to the assessment and management of adult patients with atopic dermatitis: A consensus document, J. Cutan. Med. Surg. 22 (2018) 3S-5S. https://doi.org/10.1177/1203475418803627.
17. Boguniewicz M, Fonacier L, Guttman-Yassky E, et al. (2018). Atopic dermatitis yardstick: Practical recommendations for an evolving therapeutic landscape, Ann. Allergy, Asthma Immunol. 120 (2018) 10-22.e2. https://doi.org/10.1016/j.anai.2017.10.039.
18. O'Brien J., Hayder H, Zayed Y, et al. (2018). Overview of microRNA biogenesis, mechanisms of actions, and circulation, Front. Endocrinol. (Lausanne). 9 (2018). https://doi.org/10.3389/fendo.2018.00402.
19. Weidner J, Bartel S, Kılıç A, et al. (2021). Spotlight on microRNAs in allergy and asthma, Allergy. 76 (2021) 1661–1678. https://doi.org/10.1111/all.14646.
20. Qiu L, Zhang Y, Do D.C, et al. (2018). miR-155 Modulates Cockroach Allergen– and Oxidative Stress–Induced Cyclooxygenase-2 in Asthma, J. Immunol. 201 (2018) 916–929. https://doi.org/10.4049/jimmunol.1701167.
21. Mohammed Z, McHale C, Kubinak J.L, et al. (2022). miR-155 is a positive regulator of FcεRI-

induced cyclooxygenase-2 expression and cytokine production in mast cells, Front. Allergy. 3 (2022). https://doi.org/10.3389/falgy.2022.835776.

22. Hammad N.M, Nabil F, Elbehedy E.M et al. (2021). Role of microRNA-155 as a potential biomarker for allergic rhinitis in children, Can. Respir. J. 2021 (2021) 1–7. https://doi.org/10.1155/2021/5554461.
23. Comer B.S, Camoretti-Mercado B, Kogut P.C, et al. (2015). Cyclooxygenase-2 and microRNA-155 expression are elevated in asthmatic airway smooth muscle cells, Am. J. Respir. Cell Mol. Biol. 52 (2015) 438–447. https://doi.org/10.1165/rcmb.2014-0129OC.
24. Ma L, Xue H, Wang F, et al. (2015). MicroRNA-155 may be involved in the pathogenesis of atopic dermatitis by modulating the differentiation and function of T helper type 17 (Th17) cells, Clin. Exp. Immunol. 181 (2015) 142–149. https://doi.org/10.1111/cei.12624.
25. Sonkoly E, Janson P, Majuri M.-L et al. (2010). MiR-155 is overexpressed in patients with atopic dermatitis and modulates T-cell proliferative responses by targeting cytotoxic T lymphocyte–associated antigen 4, J. Allergy Clin. Immunol. 126 (2010) 581-589.e20. https://doi.org/10.1016/j.jaci.2010.05.045.
26. Rajka G, Langeland T (1989). Grading of the severity of atopic dermatitis., Acta Derm. Venereol. Suppl. (Stockh). 144 (1989) 13–4. https://doi.org/10.2340/000155551441314.
27. Severity scoring of atopic dermatitis (1993). the SCORAD index. Consensus Report of the European Task Force on Atopic Dermatitis., Dermatology. 186 (1993) 23–31. https://doi.org/10.1159/000247298.
28. Bernstein I.L, Li J.T, Bernstein D.I, et al. (2008). Allergy diagnostic testing: an updated practice parameter., Ann. Allergy. Asthma Immunol. 100 (2008) S1-148. https://doi.org/10.1016/s1081-1206(10)60305-5.
29. Wang X, Chen Y, Yuan W et al (2019). MicroRNA-155-5p is a key regulator of allergic inflammation, modulating the epithelial barrier by targeting PKIα., Cell Death Dis. 10 (2019) 884. https://doi.org/10.1038/s41419-019-2124-x.
30. Livak K.J, Schmittgen T.D (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method., Methods. 25 (2001) 402–8. https://doi.org/10.1006/meth.2001.1262.
31. Faul F, Erdfelder E, Lang A.-G, et al. (2007). A flexible statistical power analysis program for the social, behavioral, and biomedical sciences, Behav. Res. Methods. 39 (2007) 175–191. https://doi.org/10.3758/BF03193146.
32. McPherson T (2016). Current understanding in pathogenesis of atopic dermatitis, Indian J. Dermatol. 61 (2016) 649. https://doi.org/10.4103/0019-5154.193674.
33. Rebane A, Akdis C.A (2014). MicroRNAs in allergy and asthma, Curr. Allergy Asthma Rep. 14 (2014) 424. https://doi.org/10.1007/s11882-014-0424-x.
34. Vigorito E, Kohlhaas S, Lu D., Leyland R (2013). miR-155: An ancient regulator of the immune system, Immunol. Rev. 253 (2013) 146–157. https://doi.org/10.1111/imr.12057.
35. Elton T.S, Selemon H, Elton S.M, et al. (2013). Regulation of the MIR155 host gene in physiological and pathological processes, Gene. 532 (2013) 1–12. https://doi.org/10.1016/j.gene.2012.12.009.
36. Alivernini S, Gremese E, McSharry C, et al. (2018). MicroRNA-155—at the critical interface of innate and adaptive immunity in arthritis, Front. Immunol. 8 (2018). https://doi.org/10.3389/fimmu.2017.01932.
37. Dunand-Sauthier I, Santiago-Raber M.-L, Capponi L et al. (2011). Silencing of c-Fos expression by microRNA-155 is critical for dendritic cell maturation and function, Blood. 117 (2011) 4490–4500. https://doi.org/10.1182/blood-2010-09-308064.
38. Bieber T, Novak N, Herrman N, et al. (2011). Role of dendritic cells in atopic dermatitis: An update, Clin. Rev. Allergy Immunol. 41 (2011) 254–258. https://doi.org/10.1007/s12016-010-8224-0.
39. Wollenberg A, Thomsen S.F, Lacour J.-P, et al. (2021).Targeting immunoglobulin E in atopic dermatitis: A review of the existing evidence, World Allergy Organ. J. 14 (2021) 100519. https://doi.org/10.1016/j.waojou.2021.100519.
40. Schulte-Herbrüggen O, Fölster-Holst R, von Elstermann M, et al. (2007). Clinical relevance of nerve growth factor serum levels in patients with atopic dermatitis and psoriasis., Int. Arch. Allergy Immunol. 144 (2007) 211–6. https://doi.org/10.1159/000103994.
41. Murat-Susić S, Lipozencić J, Zizić V, et al. (2006). Serum eosinophil cationic protein in children with atopic dermatitis., Int. J. Dermatol. 45 (2006) 1156–60. https://doi.org/10.1111/j.1365-4632.2006.02865.x.