Determination of changes in the expression of miR-212 and EGFR genes in clinical specimens of dermatophyte infections with *Trichophyton rubrum* in affected areas in comparison with adjacent healthy spots

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Background: Skin infections with dermatophytes are human common infections called Tinea. The most common cause of the Tinea in the world is *Trichophyton rubrum*. Antimicrobial peptides (AMPs), such as hBD-3 and RNase 7, are potent molecules in the inherent immunity of the skin, which potentially have anti-microbial effects and quickly eliminate microorganisms, which themselves are affected by the Epidermal Growth Factor Receptor (EGFR) gene and by increasing the expression of this gene in skin cells activates and prevents cloning of organisms, including dermatophytes, in keratinocytes. However, with the increase in the expression of microRNAs and in particular miR-212 in the study, the EGFR gene mRNA is muted. Two important activities of microRNA are given below:

In the first mechanism, RISC-coupled microRNAs pair up to the autolougous 3'UTR mRNA and control the gene transcription after its expression by cutting or inhibiting the target mRNA translation (Fig.1, part A and B).

In another mechanism, translation inhibition occurs with the enclosing of mRNA in processing bodies (P-bodies) (Fig.1, part C). Therefore, mRNA is not translated into proteins.

Materials and Methods: The number of specimens considered in this study was 72: 36 infected specimens with *T. rubrum* and 36 control specimens from healthy margins of the lesion (with about 15 to 20 cm) of the same patients. All specimens were immediately transferred to the -70 °C freezer. The fungus was cultivated on Sabouraud's Dextrose Agar (SDA) medium and slide culture technique was performed to observe the fungal reproductive system (Fig. 2). Extraction and optimization of total RNA and synthesis and optimization of cDNA for EGFR and miR-212 genes were performed. Amplification of these target genes and internal control genes (GAPDH and miR-103a-3p) was performed on Real-Time PCR (SYBR green method). In data aggregation and analysis, changes in target genes expression were calculated with $2^{-\Delta\Delta C_t}$ ratio. For data analysis, correlation coefficient tests and, if necessary, paired t-test were used. *P* value was considered <0.05.

Results: The amplification curve of the internal control gene or reference gene used in this study, which are called GAPDH and miR-103a-3p with the discussed genes, EGFR and miR-212, represent the results of the Real-Time PCR test that are as follows (Fig. 3 and 4).

by using the $2^{-\Delta\Delta C_t}$ formula via Excel software, the *p* value = 0.0001, which showed meaningful changes (Dia. 1).

In tissue samples infected with *T. rubrum*, miR-212 reduces expression of EGFR gene significantly and in these samples, the expression of miR-212 gene compared to the expression of EGFR gene shows 8 times increasing. In control tissue sample, the expression of miR-212 is much lower than EGFR gene expression.

Conclusion: By increasing the expression of miR-212 in this study, the mRNA function of the EGFR gene has been turned off, which this EGFR expression's reduction causes reduction of the AMPs that leads to colonization of *T. rubrum* dermatophyte and creation of Tinea on the skin.

Figure 1: Mechanism of translation inhibitor of target genes by microRNA via

Figure 2: Total RNA extracted from clinical specimens on Agarose gel 1% image

Figure 3: The amplification curve of EGFR and GAPDH internal control gene

Figure 4: The amplification curve of miR-212 gene and miR-103a-3p internal control gene

Diagram 1: Relative rate of changes in expression of EGFR and miR-212 genes in infected *Trichophyton rubrum* skin cells and their normal margin of the observed lesions

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