

Expression of MicroRNA-224-3p in vitiligo

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Abstract

Background Vitiligo is a skin and less common hair disease characterized by decline melanocytes function and depigmentation with a prevalence of 0.5–1% in most populations. MicroRNAs (miRNAs) are small single stranded; approximately 22 nucleotide-long, non-coding gene-regulatory RNA molecules that inhibit gene expression through translational repression or mRNA turnover.

Objective This case control study aimed at detection of the expression of microRNA-224-3p in lesional skin of vitiligo patients as compared to normal control persons to detect the possible role of microRNA-224-3p in the pathogenesis of vitiligo.

Methods This study included 60 patients with vitiligo and 60 age, gender matched healthy controls. 4 mm punch skin biopsy had been taken from patients (vitiligo lesion) & controls and it had been kept in lysis solution for the stability of the studied parameters and was kept frozen at -80 Celsius till analysis of microRNA-224-3p by qRT PCR.

Results The level of microRNA-224-3p in lesional skin of vitiligo was significantly higher as compared to normal control persons.

Conclusion G.

Key words

Vitiligo; MicroRNA-224-3p, polymerase chain reaction.

Introduction

Vitiligo is a skin and less common hair disease characterized by decline the function of melanocytes and depigmentation with a prevalence of 0.5–1% in most populations.¹ Studies pointed to the role of genetic susceptibility to vitiligo.² The theories have been showed combining biochemical, immunological and environmental events, in a genetic milieu.¹

MicroRNAs (miRNAs) are small single stranded; approximately 22 nucleotide-long, non-coding gene-regulatory RNA molecules that inhibit gene expression through translational repression or mRNA turnover.³

MiRNA-224-3p was identified to be correlated with a reduction in the expression of tumor necrosis factor- α , keratinocyte-derived chemokine and macrophage inflammatory protein-2, suggesting a potential role for miRNA-224-3p in the regulation of inflammatory cytokine production.⁴

The goal of this study is to detect the expression of microRNA-224-3p in lesional skin of vitiligo

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patients as compared to the normal control persons to detect the possible role of microRNA-224-3p in the pathogenesis of the disease.

Materials and subjects

This case control study included 60 patients with non segmental vitiligo and 60 age and sex matched healthy controls. The patients and controls were of Fitzpatrick skin type IV recruited from individuals attending the outpatient clinic of Beni-Suef University Hospital in the period from (1st January 2019 to 31st of June 2019).

Exclusion criteria included use of any drugs which could affect the outcome of our study as topical or systemic vitiligo treatment.

Patient information was collected by one dermatologist including age, sex, duration, type of vitiligo, affected body surface area (BSA) according to the rule of nines.¹¹

4 mm punch skin biopsy had been taken from patients (vitiligo lesion) & control and it had been kept in lysis solution for the stability of the studied parameters and had been kept frozen at -80 Celsius till analysis of microRNA-224-3p by qRT PCR for estimation of the level of microRNA-224-3p.

The aim of the study was explained to each patient, the study was approved by the local research Ethics Committee of the Faculty of Medicine, Beni-Suef University.

MiRNA-224-3p detection in tissue using qRT PCR

RNA extraction Total RNA has been isolated using Qiagen tissue extraction kit (Qiagen, USA) according to instructions of manufacture then 30 mg of the human tissue sample was

excised and placed directly into a suitably sized vessel for disruption and homogenization. The tissue was disrupted, lysed in lysis Buffer RLT and the lysate was homogenized by tissue homogenizer for 40 seconds. The lysate was centrifuged for 3 minute at full speed and the supernatant was carefully removed and transferred into a suitable microcentrifuge tube. One volume (350 µl) of 70% ethanol was then added to a cleared lysate. Seven-hundred µl of the sample was transferred to an RNeasy spin column placed in a suitable 2 ml collection tube and centrifuged for 15 sec. at ≥ 8000 rpm. Five-hundred µl Buffer RPE was then added to RNeasy spin column, and centrifuged for 15s at (≥ 8000 rpm) to wash the spin column membrane. RNeasy spin column had been then placed in a new suitable 1.5 ml collection tube. 30–50µl. RNase-free water had been added directly to spin column membrane, and then centrifuged for 1 min at (≥ 8000 rpm) to elute RNA. The eluate was then transferred to a suitable new Eppendorf tube and then stored at –80 °C for further use, purity (A260/A280 ratio) and concentration of RNA had been obtained by the spectrophotometry (dual wave length Beckman, Spectrophotometer, USA).

cDNA synthesis Total RNA (0.5–2µg) was used for cDNA conversion by using the high capacity cDNA reverse transcription kit Fermentas, USA). One µl of the random primers had been added to 10µl of RNA that had been denatured for 5 minutes at 65°C in a thermal cycler. RNA primer mixture had been then cooled to 4°C, cDNA master mix had been prepared according to kit instructions and was then added (for each sample) , last mixture was then incubated in programmed thermal cycler one hour at 42°C followed by the inactivation of the enzymes at 95°C for 10 minutes, finally cooled at 4°C. RNA had been changed to cDNA. The converted cDNA then stored at –20 °C.

Table 1 Dermographic data, Clinical characteristics of the vitiligo patients and controls.

	Patients(n=60)	control(n=60)
Gender n(%)		
Males	16 (26.7)	14 (23.3)
Females	44 (73.3)	46 (76.7)
Age (Years) Mean±SD	36.2 ±10.5	34.2 ±9.61
Duration of vitiligo (Years) Mean±SD	5.40 ±7.6	-
Family history of vitiligo n (%)		
Negative	40(66.7)	-
Positive	20(33.3)	-
History of stress at onset n (%)		
Negative	44 (73.3)	-
Positive	16 (26.7)	-

SD: Standard deviation.

Real-time qPCR using SYBR Green I Real-time qPCR amplification and analysis had been performed using Applied Biosystem with software version 3.1 (StepOne Plus™, USA). Then qPCR assay with primer sets had been optimized at annealing temperature.

Statistical analysis

Data had been coded and entered using statistical package SPSS (Statistical Package for Social Sciences) version 24. Data had been summarized using mean, standard deviation in the quantitative data and using frequency (count) and relative frequency (percentage) for the categorical data. Comparisons between the quantitative variables were then done using non-parametric Kruskal-Wallis and Mann-Whitney tests (Chan, 2003a). Correlations between the quantitative variables had been done using the Spearman correlation coefficient (Chan, 2003b). P-values less than 0.05 were considered as statistically significant.

Results

The gender ratio, age were not substantially different for each variable among patients with vitiligo (44 women, 16 men, mean±SD age 36.2±10.5 years) and healthy controls (46 women, 14 men; mean±SD age 34.2±9.61

Years. Clinical data of participants are presented in (Table 1).

The tissue microRNA-224-3p expression The level of microRNA-224-3p in lesional skin of vitiligo was significantly higher as compared to normal control persons (p-value < 0.001); where the mean±SD were (5.44±2.40 vs. 1.01±0.1) in the cases and controls respectively (Table 2, Figure 1).

We found no relation between patient age, sex, disease duration, family history, vitiligo type,

Table 2 Tissue level of microRNA-224-3p.

	Patients (n=60)	Controls (n=60)
microRNA-224-3p level [Mean±SD]	5.44 ±2.4	1.01 ±0.1

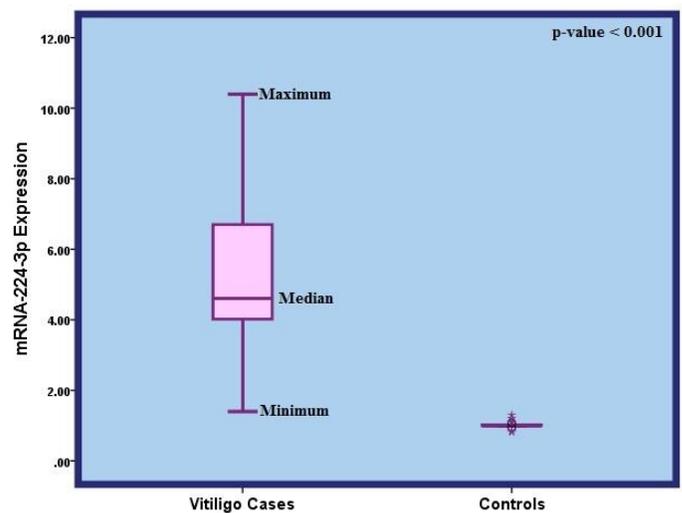


Figure 1.

affected BSA with the microRNA-224-3p expression and p-value > 0.05 in all.

Discussion

The exact pathogenesis of vitiligo is unknown but researchers suggested that an interplay of many factors such as genetic, neural, oxidant-antioxidant, biochemical, minerals and autoimmune process may induce vitiligo disease.⁵

Vitiligo melanocytes are more sensitive to accumulated reactive oxygen species (ROS) due to the intrinsic anti oxidant defects⁶ This imbalance between pro-oxidants and anti-oxidant state can disrupt the homeostasis of melanocytic cells, causing the accumulation of multiple oxidized and damaged proteins or organelles leading to destruction of melanocytes.⁷

MicroRNAs (miRNAs) are a class of small non-coding RNA molecules that regulate gene expression. A growing number of studies have shown that miRNAs modulate cellular differentiation, proliferation and apoptosis, including immune cell and melanocyte development and function. MiRNAs have been shown to be unusually well preserved in serum or plasma derived from immune cells and other tissues and serve as promising biomarkers for different diseases.⁸

All the above mentioned prompted us to investigate the role of microRNA-224-3p in vitiligo pathogenesis by detection of its tissue level in skin biopsies from vitiligo patients.

Our results showed that the level of microRNA-224-3p in lesional skin of Vitiligo disease was significantly higher as compared to the normal control persons (p-value < 0.001).

Various studies were conducted in an attempt to

detect value of microRNA-224-3p in vitiligo pathogenesis. For example *Shi et al.* performed serum miRNA expressions profiles in 10 patients with vitiligo and 20 healthy controls by microRNA arrays; they provided the first comprehensive analyses of global serum miRNA expression profiles in patients with vitiligo and had discovered distinct serum microRNA expressions during the vitiligo development.⁹

Also Wang et al found up regulation of miR-224-3p in peripheral blood mononuclear cells of patients with vitiligo.¹⁰

Among the limitations of this study is likely that there are still a lot of gaps in the knowledge base that need to be filled and to the best of our knowledge, most of the published papers detect the expression of miR-224-3p in peripheral blood from vitiligo patients; based on these results, Additional studies on large number of cases in association with assessment of miRNA-224-3p & other miRNAs to determine its exact role in pathogenesis of vitiligo.

Conclusion and Recommendations

MiR-224-3p may play a role in pathogenesis of vitiligo; hence, it can be used as a biomarker to evaluate their progression and effect of therapeutic interventions.

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