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Assessment of Genomic Micro RNA 337 biomarker in Egyptian Patient with Vitiligo

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Abstract:

Background: Inherited genes and environmental factors play several roles in the etiology of vitiligo, a common skin depigmenting condition. For a long time, scientists have been unable to pin down the exact chemical mechanism that sets off the pathological process of vitiligo. **Objectives:** to identify miRNA337's function as a novel vitiligo marker. **Methods:** In the dermatology clinic at Beni-Suef University Hospital in Egypt conducted a case control study on 52 people, splitting them evenly between 26 people with vitiligo and 26 people without the condition. Cases with vitiligo went through personal history, physical examination, vitiligo severity index (VASI) score and blood samples in both groups. Quantitative real-time polymerase chain reaction analysis (PCR) was used to compare miRNA337 concentrations between the two groups.

Results: MiRNA 337 was downregulated in the blood by a factor of two in patients compared to controls. MiRNA 337 levels in females' blood were somewhat greater than those in men', but this difference did not reach statistical significance. There was no association between VASI score, familial history, or precipitating circumstances and miRNA 337 levels in the blood. **Conclusion:** There was an inverse relationship between miRNA 337 levels and VASI score, suggesting that miRNA 337 was downregulated in vitiligo.

1. Introduction:

Clinically, vitiligo manifests as a loss of skin pigmentation over time in the form of white spots and patches caused by the death of melanocytes in the epidermis. Vitiligo has a complicated etiology that is triggered by a combination of hereditary factors and environmental stimuli. However, the chemical process that leads to vitiligo is still unclear [1].

In people of darker skin tones and in certain cultures, where vitiligo is mistaken for leprosy or another infectious skin condition, it may have a significant impact on quality of life, self-esteem, marriage, and work. Patients may fear about their racial identity if they lose their color [2].

Multiple hypotheses have been put out to explain the reasons of pigmentation loss in recent years. These hypotheses include autoimmune dysfunction, neurotoxic factor degradation, hereditary variables, and many more. Genetic polymorphisms and noncoding RNAs have recently been investigated for their potential significance in the development of vitiligo. Single-nucleotide polymorphisms (SNPs), multi-nucleotide insertions/deletions/inversions, and chromosomal translocations are all examples of genetic polymorphisms [3].

Human microRNAs (miRNAs) are noncoding, short RNAs that play critical roles in a wide variety of physiological and developmental processes. Several studies have shown that miRNAs have a regulatory role in key parts of the pathological process of vitiligo, including melanocyte proliferation, differentiation, and death, as well as the immunological response.

MicroRNAs are a subset of noncoding RNA molecules (ncRNAs) that target mRNA to control gene expression. MicroRNAs regulate gene expression by binding with partial complementarity to sequences in the 3' untranslated region (3'-UTR) of target mRNAs [4].

Multiple disorders have been linked to microRNA-337. MicroRNA-337, for instance, has been shown to have a role in modulating gene transcription and expression during liver cell differentiation. MicroRNA-337 drives gastric cancer growth. Matrix metalloproteinase-14 (MMP-14) expression is suppressed by microRNA-337, which in turn slows the development of neuroblastoma [5].

Moreover, microRNA-337's function in osteoarthritis, melanoma, and pancreatic cancer has been documented. Unfortunately, microRNA-337's involvement in Vitiligo has not been investigated. To offer a theoretical foundation for the diagnosis and treatment of Vitiligo, understanding the role of microRNA-337 is crucial [6]. The aim of the current study was to evaluate gene expression of microRNA 337 in patients with vitiligo as biomarker for diagnosis and pathogenesis of the disease

2. Patients and methods:

2.1. Participants and study design:

This was a Case control study took place at dermatology department in Beni-Suef University hospital. Patients were enrolled into the study starting from February 2022 to be continuing till reaching the target number of cases based on sample size calculation. The study included two matched groups: Group (1): A sample of 26 eligible subjects had been required as vitiligo cases group. Group (2): An equal number of healthy subjects; 26 in a ratio of 1:1 had been required in the control group Patients and controls were selected according to the following inclusion criteria as; Patients with vitiligo, regardless of their age or gender, were considered for inclusion in the study. Patients of the same age and gender were randomly assigned to our healthy control group. While; patients undergoing phototherapy, Patients who are on vitiligo treatment (such as immunosuppressive drugs), patients with chronic diseases (such as diabetes, hypertension), Immuno-compromised patients, patients whose conditions are diagnosed as cutaneous tumors, and patients who are in need of immediate medical and surgical attention were excluded from the study. This study was greenlit by Beni-Suef University's

medical school's ethical committee. All people were told and given signed consent before we began recruiting for the research. This was carried out after a comprehensive explanation of the study objectives. The administration of the database was done with the utmost secrecy. Ethical approval number is: FMBSUREC/09012022/Emam.

2.2. Methods:

A comprehensive history was taken from each participant, including the following information: Age (years), Vitiligo onset (Sudden, Gradual), How Long Does the Vitiligo Disease Last in months, The Vitiligo Disease's Natural Progression (Progressive, Regressive or Stationary), A history of the vitiligo disease in the family (Positive and negative), and vitiligo's risk factors and precipitating conditions. Evaluation in the clinic was to establish the kind, magnitude, and distribution of vitiligo. The Vitiligo Area and Severity Index, abbreviated as VASI, was calculated for each individual instance of vitiligo. This index is a tool for determining the severity and extent of vitiligo. The total body VASI is calculated using a formula that includes contributions from all body regions (possible range, 0–100) [7].

Blood samples used to assay the miRNA- 337 level in the sample using All-in-One™ miRNA qRT-PCR Detection Kit 2.0 for quantitative detection of mature miRNA, Cat. No. QP116. Used in combination with the All-in-OneTM miRNA qPCR primers Purchased from Gene Copoeia, Inc. 9620 Medical Center Drive, #101 Rockville, MD 20850; USA, as shown in table (1).

Table (1): Primers

	U6 (Internal control)	**miRNA-337**
Forward,	5′-CTCGCTTCGGCAGCACA-3′	5′-ACA CTC CAG CTG GGC TCC TAT ATG ATG C-3′
Reverse,	5′-AACGCTTCACGAATTTGCGT-3′	5′-ACT CCA CGA CAC CAG TTG AG-3′

2.3. Statistical analysis:

SPSS version 25 (Statistical Package for Social Science) on Windows 10 was used to code, handle, and analyze the data.

There were the following tests used: A percentage distribution for the qualitative data and find the minimum, maximum, mean, and standard deviation (SD) for the numeric data were to analyze the results. To compare group data and find the percentages, cross-tabulation and the Chi Square test ($\chi 2$) were used, to compare the means of two separate groups that follow a normal distribution, you can use the Student t-test. Another way to compare the means of many groups that are not linked is with the One-way ANOVA test. We used Pearson's correlation analysis to check if there was a straight line link between the amount of miRNA-337 and other factors in skin biopsies and plasma samples from people who have Vitiligo. There were only relationships that were given a correlation graph if they were statistically significant ($P < 0.05$). When the correlation coefficient (r) has a positive sign, it means that the correlation is positive (direct correlation). When it has a negative sign, it means that the correlation is negative (inverse correlation). If r is between 0 and 0.35, the correlation is weak. If r is between 0.35 and 0.65, the correlation is moderate. And if r is more than 0.65, the correlation is strong. For something to be statistically significant, the p-value had to be equal to or less than 0.05.

3. Results:

Table 2 shows that; 46.2% of vitiligo patients were males and 53.8% were females. There was no statistically significant difference between cases and control groups regarding sex (p=0.781) and age (p=0.082)

Table (2): Basic demographic data of study participants:

		N (%)		TOTAL	p-value
		Vitiligo Cases N= 26	Healthy Controls N= 26		
Sex	**Male**	12 (46.2%)	13 (50%)	25 (48.1%)	0.781
	Female	14 (53.8%)	13 (50%)	27 (51.9%)	
Age	<30 y	30-40 y	>40 y	Total	p-value
Vitiligo Cases (N)	16	9	1	26	0.082
Healthy Controls (N)	10	10	6	26	

Table (3) shows that, Stress as a predisposing factor of vitiligo in the studied cases represented (30.8%), followed by anxiety in (7.7%) of cases while, in 61.5% of vitiligo cases the predisposing factors were unknown. The majority of cases reported no family history of vitiligo disease (88.5%) while only (11.5%) had positive family history of vitiligo. The mean duration of vitiligo disease among the cases recorded were (10.96 ±10.19) years. The majority of vitiligo cases (61.5%) have generalized lesions in the whole body while 19.3% of the patients have mixed lesions. 7.7% of the cases have acral and localized lesions in the lower limbs, also 3.8% of the cases have facial lesions.

Table (3): Basic clinical data of vitiligo patients:

Precipitating Factors (N/%)	
Stress	8 (30.8%)
Anxiety	2 (7.7%)
Unknown	16 (61.5%)
Family History (N/%)	
Positive	3 (11.5%)
Negative	23 (88.5%)
Duration (Years) Mean ±SD Minimum Maximum	10.96 ±10.19 1 40
Vitiligo Areas (N/%)	
Acral	2 (7.7%)
Facial	1(3.8%)
Generalized	16 (61.5%)
Localized	2 (7.7%)
Mixed	5 (19.3%)

In table (4): MiRNA 337 levels in vitiligo group were significantly decreased when compared with the control group. Highly statistical difference was recorded between both groups (p<0.001**)

Table (4): Serum level of Fold change miRNA 337 among vitiligo patients as compared with healthy controls; (N= 52):

	Vitiligo Cases N= 26	**Healthy Controls N= 26**	***p-value***
Mean ±SD	0.61±0.51	1.63±0.30	<0.001**
Range	1.64	1.10	
Minimum	0.13	1.10	
Maximum	1.77	2.20	

** P-value ≤ **0.05** is considered significant by (T-test).*

In table (5): Serum levels of miRNA 337 were slightly higher among females as compared with males, however this difference showed non-statistically significant value. Serum levels of miRNA 337 showed non-statistically significant difference in relation to family history of vitiligo disease (p-values >0.05), and precipitating factors (p-values >0.05).

Table (5): Relation between serum level of miRNA 337 and basic data in studied vitiligo patients; (N= 26):

Serum Level miRNA		**N**	**Mean**	**SD**	**Min.**	**Max.**	**p-value**
Sex	Male	12	0.54	0.52	0.22	1.76	0.500 (NS)
	Female	14	0.68	0.51	0.13	1.77	
Family history	Negative	23	0.63	0.53	0.16	1.77	0.465 (NS)
	Positive	3	0.40	0.34	0.13	0.78	
Precipitating factors	Stress	8	0.43	0.24	0.13	0.78	0.499 (NS)
	Anxiety	2	0.76	0.01	0.75	0.77	
	Unknown	16	0.68	0.62	0.13	1.77	

** P-value ≤ **0.05** is considered significant by (T-test).*

In table (6): shows no correlation between serum levels of miRNA 337 with VASI score in vitiligo cases (r=0.171, p=0.403). No linear correlations were recorded between the studied parameters.

Table (6) Correlation of Fold Change miRNA 337 and VASI score with age and Disease duration studied parameters in Vitiligo group.

Variable	miRNA 337		VASI score	
	r	**p-value**	**r**	**p-value**
miRNA 337	-	-	0.171	0.403
Age	-0.315	0.117	-0.146	0.486
Disease Duration	-0.205	0.314	0.2	0.337

r=Pearson's correlation coefficient

4. Discussion:

A depigmenting disorder known as vitiligo affects between 0.1 and 2% of the world's population. The noticeable loss of melanocytes is the root cause of the white patches. A thorough understanding of the genetic architecture of vitiligo has been achieved via recent and continuing work. Environmental factors account for about 20% of the risk of vitiligo, whereas genetic factors account for over 80% [8].

Current evidence suggests that gene interactions, particularly those involving noncoding RNAs, contribute to an individual's susceptibility to vitiligo and may even be responsible for its onset and progression. miRNAs are small noncoding RNAs with a negative regulatory role; they are around 22 nucleotides long. They do this by attaching to the 3′-UTR or 5′-UTR sections of the target mRNA, which causes translation to be halted or mRNA degradation to begin. To better understand how vitiligo develops, Yan et al. [9] performed research that reveals the function of microRNAs and regulatory pathways. Furthermore, it offers patients hope that tiny molecules may one day be employed as a treatment for vitiligo.

Examining MicroRNA 337's potential as a biomarker for Vitiligo diagnosis and progression was the driving force for our study. This study compared a control group of people without vitiligo to see how they were distributed by gender. Males made for 46.2% of the vitiligo patients, while females

accounted for 53.8%. The gender distribution of the cases and controls did not vary significantly (p=0.781).

Everyone, regardless of race, gender, or skin tone, is equally susceptible to vitiligo. Despite this, medical consultations are more often sought by women and girls [10]. It was shown in a review article on vitiligo by Bergqvist and Ezzedine [11] that the illness affects men and females equally. On the other hand, males and boys are less likely to seek advice than women and girls, who may be more affected by the social stigma [12].

In this study, we compare a group of healthy controls against those who have vitiligo and look at their age distribution. The age distribution of the patients and controls did not vary significantly (p=0.082).

Worldwide, vitiligo affects an estimated 0.1% to 2% of the adult and pediatric population, making it the most common cause of depigmentation [13]. According to Nicolaidou et al. [14], vitiligo may emerge at any age, although it is more common in people between the ages of 10 and 30. According to Jin et al. [15], there are two separate peaks in the frequency of specific phenomena, and many populations show a mixture of many age groups at which it starts .

These findings suggest that stress may have a role in the development of vitiligo in 30.8% of patients. Seven point seven percent of the patients had anxiety as an underlying factor. The factors that put people at risk for vitiligo were not clearly defined in 61.5% of case. Sunlight and skin damage are known to cause oxidative stress in melanocytes and autoimmune responses mediated by T cells, which may lead to vitiligo [16]. Physical or environmental pressures are linked to the onset and progression of vitiligo [17]. Mental health issues might contribute to vitiligo. Additionally, vitiligo has major psychological effects, such as increased anxiety, depression, social embarrassment, and a worse quality of life for those who suffer from it. Henning et al. [16] found that stress is a factor in the development of vitiligo since people with the condition had higher levels of cortisol, neuropeptides, and catecholamines.

This study looked at the possibility of vitiligo running in families among the participants. A minor percentage (11.5%) of patients had a positive family history of vitiligo, whereas the vast majority (88.5%) did not have any documented cases in their family history .

In line with our results, Pajvani et al. [18] found that vitiligo is more common in younger people with a familial history of the disorder than in those without such a history. The location, distribution, or course of the disease was not correlated with family history. By comparing vitiligo patients to a population-based control group, Nejad and colleagues [19] were able to ascertain the prevalence of autoimmune conditions in the former. A familial history of vitiligo was reported in 24% of vitiligo patients in the research. Agarwal et al. [20] found that a family history of vitiligo may explain why certain cases manifest in children at a young age. According to our study, the subjects' average vitiligo duration was 10.96 ± 10.19 years .

Research was carried out to determine the effect of sickness duration on the clinical response of Vitiligo to NB-UVB phototherapy based on participant comments. Of the patients, 26 developed the illness very recently, whereas 37 had it for a long time. According to Hallaji et al. [21], the mean length of sickness was 10.13 ± 9.1 years. Silpa-Archa et al. [22] found that the average age of vitiligo onset was 40.7 years (range: 3-70), which is different from our people.

According to the study, 61.5% of patients had systemic lesions, while 19.3% of patients had a mix of different kinds of lesions. Acral and localized lesions in the lower limbs are seen in 7.7% of patients, whereas facial lesions are observed in 3.8% of cases .

Also, out of 681 medical records, 61.4% were classified as having non-segmental vitiligo (NSV), with 52.2% of those cases being generalized vitiligo. There are many types of vitiligo, with 29.5% being segmental vitiligo (SV), 5.7% being focal vitiligo, 4.6% being acrofacial vitiligo, 2.3% being mucosal vitiligo, 1.9% being mixed vitiligo, and 1% being universal vitiligo. Starting in 44.2% of instances, the head and neck area was the most severely affected area. While 55.9% of patients with NSV had Koebner's phenomenon, only 10.6% of those with segmental vitiligo and 6.4% of those with ambiguous vitiligo did so [23].

In our study, we compared the descriptive statistics of the vitiligo group to those of the healthy control group using data acquired from both groups. With a standard deviation of 1.50, the average VASI score was 2.08. The range of values reported ranged from 0.2 to 5.5. A mean value of 2.08 was computed.

Mogawer et al. [24] investigated vitiligo patients using a cross-sectional study that compared the Vitiligo Extent Score to the VASI score. The VASI score had an average value of 7.59 ± 12.17. There are a variety of methods for evaluating vitiligo, including subjective, semi-objective, and objective processes [25], which might explain why our results don't match up with others .

We found a significantly significant difference between the vitiligo and healthy groups when we compared the levels of fold change micRNA 337. The vitiligo group showed significantly lower expression of miRNA 337 compared to the control group. While there was a statistically insignificant difference between the sexes, women did have slightly higher blood levels of miRNA 337 than men. There was no statistically significant correlation between vitiligo patients' blood levels of miRNA 337 and their familial history of the disease. No statistically significant difference was seen in the blood levels of miRNA 337 in relation to vitiligo disease triggering factors, age, or sickness duration. Patients with vitiligo do not show a statistically significant link (r=0.171, p-value=0.403) between blood levels of miRNA 337 and the VASI score .

To the best of our knowledge, no prior studies have investigated genomic miRNA 337 in individuals with vitiligo. Nevertheless, a genetic disturbance of the immune system's monitoring of the melanocytic system was suggested by Spritz and Andersen [26], who discovered a clear correlation between vitiligo and melanoma susceptibility. According to research by Xiao and colleagues [27], miRNA-337 expression was lower in melanoma tissues when compared with nearby tissues. At the same time, compared to nearby tissues, melanoma tissues showed an approximately 20% increase in STAT3 expression. Downregulation of STAT3 by miRNA-337 was the mechanism by which miRNA-337 suppressed melanoma cells.Vitiligo skin had significantly higher H-scores for STAT3 expression in the dermis and epidermis than the other groups, according to research by Samaka et al. [28]. Our results could be explained by this observation, which points to a possible mechanism. In order to determine how miR-337 regulates CTCL cell viability and invasion, Xia et al. [29] performed a molecular analysis. The results show that the miR-337-STAT3 pathway inhibits the proliferation of malignant T cells,

suggesting that miR-337 might be a therapeutic target in CTCL. Our study suggests that miRNA 337 may have a role in Vitiligo progression and disease severity assessment.

5. Conclusion:

A link between vitiligo and high serum levels has been found in the present study. Although there was no correlation between the down-regulated levels of miRNA 337 in vitiligo and either the VASI score or the etiology of vitiligo, more research with bigger samples is needed to confirm or disprove its significance.

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