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# ASSOCIATION BETWEEN MICRORNA 196A-2 POLYMORPHISM AND SERUM TYROSINASE LEVEL AND ITS INFLUENCE ON TREATMENT OF VITILIGO PATIENTS

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## ABSTRACT

**Background:** MicroRNAs (miRNAs) are discovered family of endogenous, noncoding RNA molecules. miRNAs modulate the gene expression post-transcriptionally by binding to complementary sequences in the coding or 3' untranslated region of target mRNAs. It was demonstrated also that miRNAs are the key regulators of a variety of biological processes such as cell proliferation, apoptosis and tumorigenesis. The miR-196a-2 could also potentially target genes such as platelet-derived growth factor receptor, alpha-polypeptide (PDGFRα), IL2, and mannose-binding lectin (protein C) 2 (MBL2) that are likely candidates of several oxidative stress-mediated diseases. In addition, miR-196a-2 was also demonstrated to target tyrosinase, which plays an important role in melanin synthesis. **The aim** of this study was to investigate the association between a functional SNP of rs 11614913 in microRNA 196a-2 and the serum tyrosinase level in vitiligo patients, evaluate the effect of microRNA 196a-2 polymorphism on the response to treatment in vitiligo patients, as well as to explore this polymorphism in first degree relatives of vitiligo patients. **Methods:** The current study was a prospective case-control interventional study that was conducted on 100 participants. Fifty of them were patients with vitiligo, and they were located in group 1. Group 2 contained 50 control participants, half of them were 1st-degree relatives for vitiligo patients (group 2a), while the rest had a negative family history. In group 1, determination of the miRNA 196a-2 polymorphism as well as assay of the level of serum Tyrosinase. Then, they were treated for 4 months by NB-UVB. Then after the end of the treatment course, serum tyrosinase level was remeasured and the response of the treatment was evaluated. Group 2 was tested also for the type of polymorphism and the level of serum Tyrosinase. **Results:** The level of serum tyrosinase is higher in patients with vitiligo. And the levels of the enzyme were significantly higher in cases suffering from early onset vitiligo. Patients with higher serum Tyrosinase level tended to be more resistant to treatment. And the treatment course of vitiligo failed to record a significant reduction in the serum Tyrosinase levels. T allele of miRNA 196a-2 polymorphism was found to have a significant relation with the development of vitiligo. And presence of TT genotype could predict the early age of onset and poor response to treatment, while C allele is associated with lower level of serum Tyrosinase level. **Conclusion:** MiRNA 196a-2 polymorphism could be used as a predictive marker for the expected development of vitiligo.

**KEYWORDS:** Vitiligo; tyrosinase; polymorphism; microRNA.

## INTRODUCTION

Vitiligo is an idiopathic skin disease characterized by selective destruction of melanocytes leading to depigmentation in the form of milky white macules or patches. Vitiligo occurs worldwide with an estimated prevalence of 0.5–1%. In almost half of the patients, vitiligo starts before the age of 20 years, however, it can be seen at any age group with no significant sex difference **(Boniface et al., 2018).**

Although the etiopathogenesis of vitiligo is not yet fully understood, the autoimmune, auto cytotoxic, neural, and biochemical-based hypotheses are considered. Genetic predisposition and triggering factors have roles in the emergence of the disease **(Iannella et al., 2016).**

Micro-RNAs contribute to the cellular regulatory processes via their capacity to alter the expression of approximately 60% human genes at both post-transcription and translation levels. Therefore, miRNAs are of great importance in diverse physiological and developmental processes in humans including development and function of melanocytes as well as immune cells **(Mansuri et al., 2016).**

Recently, miRNA-196-a2 gained a lot of attention. It has been reported to be deregulated in various cancer types

and consequently, this up- or down-regulation may impact tumor malignancy or drug resistance according to the downstream target genes it affects. Bioinformatics analysis had shown that miR-196-a2 could target many genes enriched in cell cycle regulation, survival and apoptosis **(Fawzy et al., 2017).**

It was found that miR-196a2 polymorphism contributes to pathogenesis of variety of diseases and is possible genetic predisposing factor. MiRNA196a2 was found to be associated with many systemic diseases such as coronary artery disease **(Buraczynska et al., 2014),** GI cancers **(Fawzy et al., 2017)**, increase the risk of ischemic stroke **(Zhu et al., 2018)**, certain types of HCC **(Hou et al., 2010).**

However, little is known regarding the contribution of genetic variations in miRNAs to the development of vitiligo **(Huang et al., 2012)**. **Manga et al. (2006)**, hypothesized that SNPs in miR-196a-2 could potentially alter regulation of the expression of the target TYRP1, an enzyme that may use an oxidative inducer as a substrate and promote ROS formation by catalyzing quinone production in melanocytes, leading to individual susceptibility to vitiligo.

In addition, **Cui et al. (2015),** also demonstrated that miR-196a-2 targets Tyrp1gene. And the rs11614913 T/C change in miRNA196a-2 could down-regulate the cellular level of ROS and protect human melanocytes from apoptosis by suppressing the expression of Tyrp1.

The aim of this study was to investigate the association between a functional SNP of rs 11614913 in microRNA 196a-2 and the serum tyrosinase level in vitiligo patients, evaluate the effect of microRNA 196a-2 polymorphism on the response to treatment in vitiligo patients, as well as to explore this polymorphism in first degree relatives of vitiligo patients.

## SUBJECTS AND METHODS

The current study was a prospective case-control interventional study that was conducted on patients with vitiligo in Benha University Hospital. This study was conducted on 100 participants. Fifty of them were patients with vitiligo, and they were located in group 1. Group 2 contained 50 control participants, twenty five out of them were 1st-degree relatives for vitiligo patients (group 2a), while the other 25 had a negative family history.

In group 1, the patients assessed with full history taking, general and dermatological examination with photographic documentation. The severity of the condition was assessed by rule of nine and VASI score. Then blood samples were taken for laboratory investigations. Determination of the miRNA 196a-2 polymorphism with PCR as well as assay of the serum level of tyrosinase enzyme using ELISA. Then, they were treated for 4 months by NB-UVB with the regimen of twice weekly sessions with the starting fixed dose of 200mJ/cm2 irrespective to skin type with increment in dose by about10-20% per session unless side effects were appearing. Then after the end of the treatment course, serum tyrosinase level was remeasured and the response of the treatment was evaluated objectively through VASI score and subjectively through estimating the patient satisfaction.

Group 2 was tested also for the type of miRNA 196-a2 polymorphism and the serum level of Tyrosinase enzyme to be compared with the first group. The participants in group 2 were selected to be matching in age and sex to group 1.

Qualitative data were summarized in the form of frequency and percentage. Mean and SD were obtained for quantitative data, while categorical data were presented by number and percentage. One way analysis of variance (ANOVA) test, paired and independent sample t-tests were used for comparing means between groups. Tests used for association were Chi square (X2) or Mont-Carlo Exact test (MCET). P -value was adopted to be <0.05.

## RESULTS

Analysis of the disease characteristics among the patients in group 1 was performed regarding the age of onset, type of vitiligo, course of the disease, associated autoimmune disease, previous lines of treatment, family history of vitiligo, surface area of the affected area (measured by rule of nine) and the severity of the condition (evaluated by VASI) **(Table 1)**.

**Table (1): Vitiligo characteristics among studied patients (n.=50).**

| TOTAL | n | % |
|---|---|---|
| | 50 | 100 |
| **Age of onset** | | |
| < 10 | 9 | 18 |
| 10 - 19 | 16 | 32 |
| 20 - 29 | 12 | 24 |
| 30 - 39 | 6 | 12 |
| ≥40 | 7 | 14 |
| **Type of vitiligo** | | |
| Vulgaris | 38 | 76 |
| Acral | 6 | 12 |
| Acrofacial | 5 | 10 |
| Focal | 1 | 2 |
| **Course of the disease** | | |
| Progressive | 22 | 44 |
| Intermittent | 14 | 28 |
| Stationary | 8 | 16 |
| Regressive | 6 | 12 |
| **Associated autoimmune diseases** | | |
| No | 41 | 82 |
| Rheumatoid arthritis | 3 | 6 |
| Thyroiditis | 2 | 4 |
| Grave's disease | 2 | 4 |
| Alopecia areata | 2 | 4 |
| **Previous lines of treatment** | | |
| Phototherapy only | 23 | 46 |
| Topical only | 7 | 14 |
| Phototherapy + topical | 12 | 24 |
| Phototherapy + topical + antioxidants | 3 | 6 |
| No previous treatment | 5 | 10 |
| **Family history of vitiligo** | | |
| Negative | 32 | 64 |
| Positive | 18 | 36 |
| **Surface area of vitiligo** | | |
| < 10% | 10 | 20 |
| 10 - <20 | 21 | 42 |
| 20 - <30 | 9 | 18 |
| ≥ 30 | 10 | 20 |
| **VASI** | | |
| ≤5 | 12 | 24 |
| >5 - 10 | 22 | 44 |
| >10 - 15 | 6 | 12 |
| >15 - 20 | 4 | 8 |
| >20 | 6 | 12 |

The degree of improvement was assessed objectively and subjectively. The objective response was evaluated by recalculation of VASI score and detecting the extent of improvement by subtraction of the pre-treatment recorded values from that recorded after treatment, then dividing the result on the original VASI score of the patient before treatment **(Table 2)**. The improvement was also assessed subjectively (by the patients themselves) through evaluation the patient satisfaction (and the degree of subjective improvement was classified into good, fair, no response and worsening) **(Table 3)**.

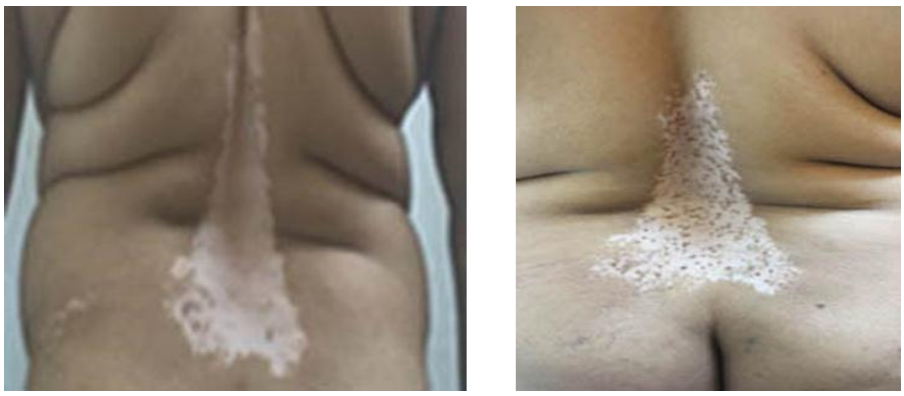
**Table (2): Degree of Objective Improvement Among Studied Patients.**

| Objective response | | | |
|---|---|---|---|
| **Code** | **Grade** | **no** | **Percentage** |
| -4 | Very much worse | 0 | 0% |
| -3 | Much worse | 0 | 0% |
| -2 | Worse | 0 | 0% |
| -1 | Minimal worse | 4 | 8% |
| 0 | Stationary | 8 | 16% |
| 1 | Minimal improvement | 20 | 40% |
| 2 | Improvement | 14 | 28% |
| 3 | Much improvement | 3 | 6% |
| 4 | Very much improvement | 1 | 2% |
| | Total | 50 | 100% |

**Table (3): Degree of Subjective Improvement Among Studied Patients.**

| Subjective Response | n | % |
|---|---|---|
| **Good** | 14 | 28 |
| **Fair** | 21 | 42 |
| **No** | 11 | 22 |
| **Worsening** | 4 | 8 |
| **Total** | 50 | 100 |

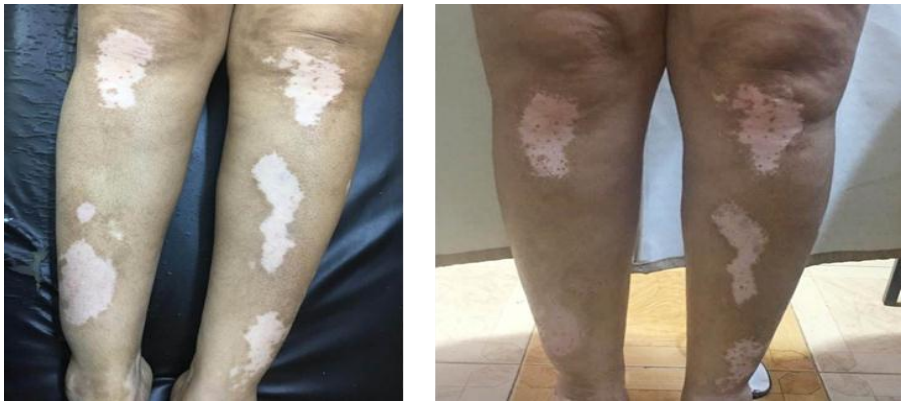
Photographs were used to document the extent and severity as well as to monitor the degree of improvement. Samples of the studied patients before and after treatment are illustrated in **(Fig. 1,2&3).**



**A** **B**

**Fig. (1): One of the studied patients with vitiligo vulgaris affecting the back.**

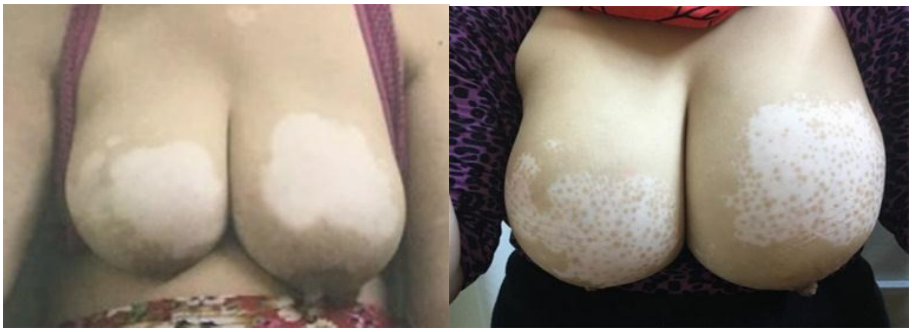
A- Before treatment: the degree of depigmentaion in the back was 90%.
B- B- The improvement after treatment for 4 months. The degree of depigmgmentaion became 75%.



**A** **B**

***Fig. (2):* One of the studied patients with acralfacial vitiligo affecting the lower limbs.**

A- Before treatment: The degree of the pigmentation was 100% in the mid-leg lesion and 90% for the knee lesions.
B- The improvement after treatment for 4 months. The repigmentaion appeared at the periphery of the lesions. The degree of depigmentation became 90% and 75% fot the mid-leg and knee lesions respectively.



***A*** ***B***

***Fig. (3):*** One of the studied patients with vitiligo vulgaris affecting the breast.

A- Before treatment: the degree of depigmentation was 100%.
B- The improvement after treatment for 4 months. The degree of depigmentation became 75%.

Serum tyrosinase level was found to be higher in group 1 (89.05±2.6) than in group 2 (80.4± 1.34). There was a statistically highly significant difference between the 2 groups (P <0.0001) **(Fig. 4)**. On the other hand, there was no statistical difference between group 2a and group 2b regarding the level of serum tyrosinase (P= 0.9).

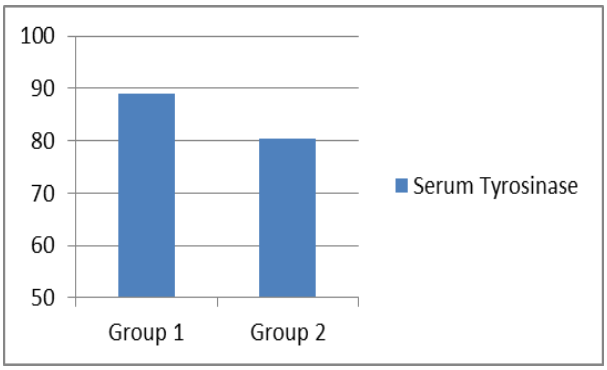



**Fig. (4): The mean serum tyrosinase level among studied groups.**

Serum tyrosinase level also was found to be slightly reduced in response to treatment (89.05 ±2.6 & 88.08±2.8), however, this reduction is not significant at all (P= 0.079).

Regarding the miRNA 196-a2 polymorphism, it was found that TT genotype was more prevalent among the diseased patients, while CC genotype was more prevalent among the control group **(Table 4)**. On the other hand, miRNA 196-a2 polymorphism failed to show any statistically significant difference between the relative and control group (P=0.9).

**Table 4: MiRNA 196a2 Polymorphism Between Cases and Controls Groups.**

| MiRNA 196a2 polymorphism | Group 1 | | Group 2 | | P value |
|---|---|---|---|---|---|
| | n | % | n | % | |
| **CC** | 21 | 42 | 31 | 62 | MCET=15.2 P=0.014* |
| **CT** | 0 | 0 | 2 | 4 | |
| **TT** | 29 | 58 | 17 | 34 | |
| **Total** | 50 | 100 | 50 | 100 | |

The relationship between the miRNA 196-a2 polymorphism and the different characteristics of the disease was studied among the included patients in group 1, and it is illustrated in **Table 5.**

The miRNA 196-a2 polymorphism was found to have a highly significant relation with both the degree of improvement either objectively (VASI score) (P <0.001*) **(Fig. 5)** or subjectively **(figure 6)**, as well as the age of onset. CC phenotype was highly related to better results, while TT genotype was related significantly to poorer results. Also, TT genotype was significantly related to earlier age of onset of vitiligo.

On the other hand the genomic polymorphism failed to demonstrate any statistically significant relation with development of associated autoimmune disease, positive family history, type of vitiligo nor with course of the disease.

**Table 5: Relationship between miRNA 196-a2 polymorphism and disease characteristics among group 1.**

| | MiRNA 196 a2 polymorphism | | | | P value |
|---|---|---|---|---|---|
| | CC | | TT | | |
| | n | % | n | % | |
| **TOTAL** | **21** | **100** | **29** | **100** | |
| **Subjective improvement** | | | | | |
| **Good** | 12 | 57.1 | 2 | 6.9 | MCET=18.9 P=0.0002* |
| **Fair** | 8 | 38.1 | 13 | 44.8 | |
| **No** | 1 | 4.8 | 10 | 34.5 | |
| **Worsening** | 0 | 0 | 4 | 13.8 | |
| **Associated autoimmune disease** | | | | | |
| **No** | 15 | 71.4 | 26 | 89.7 | MCET=4.11 P=0.39 |
| **Rheumatoid arthritis** | 2 | 9.5 | 1 | 3.4 | |
| **Thyroiditis** | 1 | 4.8 | 1 | 3.4 | |
| **Grave's disease** | 2 | 9.5 | 0 | 0 | |
| **Alopecia areata** | 1 | 4.8 | 1 | 3.4 | |
| **Family History** | | | | | |
| **Negative** | 15 | 71.4 | 17 | 58.6 | X2=0.87 P=0.35 |
| **Positive** | 6 | 28.6 | 12 | 41.4 | |
| **Age of onset** | | | | | |
| **< 10** | 0 | 0 | 9 | 31 | MCET=22.1 P=0.0001* |
| **10 - 19** | 3 | 14.3 | 13 | 44.8 | |
| **20 - 29** | 7 | 33.3 | 5 | 17.2 | |
| **30 - 39** | 6 | 28.6 | 0 | 0 | |
| **≥40** | 5 | 23.8 | 2 | 6.9 | |
| **Type of vitiligo** | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| **Vulgaris** | 15 | 71.4 | 23 | 79.3 | MCET= 1.64 P=0.6 |
| **Acral** | 3 | 14.3 | 3 | 10.3 | |
| **Acrofacial** | 3 | 14.3 | 2 | 6.9 | |
| **Focal** | 0 | 0 | 1 | 3.4 | |
| **Course of the disease** | | | | | |
| **Progressive** | 10 | 47.6 | 12 | 41.4 | X2=1.15 P=0.16 |
| **Intermittent** | 4 | 19 | 10 | 34.5 | |
| **Stationary** | 4 | 19 | 4 | 13.8 | |
| **Regressive** | 3 | 14.3 | 3 | 10.3 | |

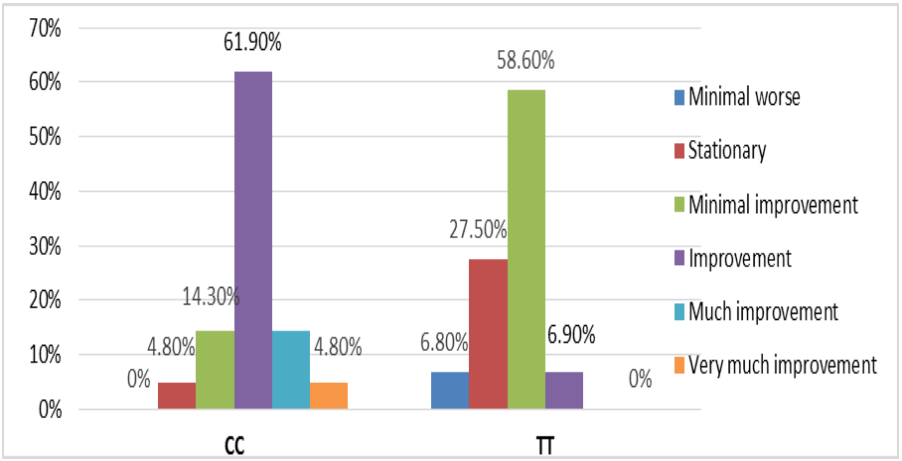



**Fig. (5): Relationship between miRNA 196-a2 polymorphism and the degree of objective improvement.**

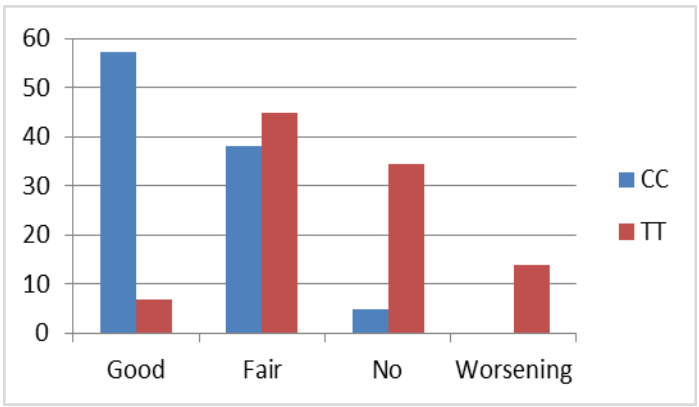



**Fig. (6): Relationship between miRNA 196-a2 polymorphism and the degree of subjective improvement.**

The miRNA 196-a2 polymorphism was significantly related to the level of serum tyrosinase. It was found that TT genotype was related to a higher level of serum tyrosinase in comparison to CC genotype **(Table 7).**

**Table (7): Association Between Polymorphism and Pre-Treatment Serum Tyrosinase Level Among Studied patients.**

| S. Tyrosinase | Polymorphism | | F | P value |
|---|---|---|---|---|
| | CC (n=21) | TT (n=29) | | |
| Mean & standard deviation | 87.18 ±2.29 | 90.4 ±1.89 | 29.6 | 0.0002* |

Serum Tyrosinase level was found not to be affected by the age or the gender of the patient. On the other hand, the level of serum tyrosinase was found to have significant relation with age of onset of the disease **(Fig. 7)** as well as the objective improvement assessed by VASI **(Table 8)** and the subjective improvement. There was no detectable significant relation with the type of vitiligo, the severity of the disease or the subjective degree of improvement after treatment.

**Table (7): Relationship between s.Tyrosinase and socio-demographic data and disease characteristics.**

| | Pre- treatment S. Tyrosinase level (mg/dl) | | |
|---|---|---|---|
| | **Mean ±SD** | **F** | **P value** |
| **Gender:** | | | |
| **Male** | 88.89 ±3.28 | 0.133 | 0.7 |
| **Female** | 89.14 ±2.3 | | |
| **Age:** | | | |
| **< 20** | 89.87 ±2.46 | 0.401 | 0.7 |
| **20-29** | 89.4± 2.9 | | |
| **30-39** | 88.49 ±2.5 | | |
| **≥40** | 89 ±2.7 | | |
| **Age of onset:** | | | |
| **< 10** | 89.06± 2.57 | 4.04 | 0.007* |
| **10 - 19** | 90.6±1.54 | | |
| **20 - 29** | 88.8±2.47 | | |
| **30 - 39** | 86.4±2.12 | | |
| **≥40** | 88.4± 3.3 | | |
| **Type:** | | | |
| **Vulgaris** | 89.3±2.4 | 1.02 | 0.3 |
| **Acral** | 87.3±3.7 | | |
| **Acrofacial** | 88.9±2.4 | | |
| **Focal** | 89.7 | | |
| **Severity (VASI)** | | | |
| **≤5** | 88.57 ±2.9 | 0.48 | 0.7 |
| **>5 - 10** | 88.9±2.59 | | |
| **>10- 15** | 90.35±2.02 | | |
| **>15 - 20** | 89.1±2.32 | | |
| **>20** | 89.25±2.93 | | |
| **Subjective response:** | | | |
| **Good** | 87.7±3.1 | 2.03 | 0.12 |
| **Fair** | 89.65±2 | | |
| **No** | 89.6±2.45 | | |
| **Worsening** | 89.62±2.29 | | |

**Table (8): Relationship Between Serum Tyrosinase and The Objective Response To Treatment.**

| Objective Response | no | S. Tyrosinase (Mean & SD) | F | P value |
|---|---|---|---|---|
| Minimal worse | 3 | 91.00 ±1.00 | 3.53 | 0.009* |
| Stationary | 8 | 89.75 ± 2.87 | | |
| Minimal improvement | 20 | 89.75 ± 2.22 | | |
| Improvement | 15 | 87.87 ± 2.29 | | |
| Much improvement | 3 | 88.67 ± 2.52 | | |
| Very much improvement | 1 | 82.00 | | |

## DISCUSSION

Micro-RNAs contribute to the cellular regulatory processes via their capacity to alter the expression of approximately 60% human genes at both post-transcription and translation levels. Therefore, miRNAs are of great importance in diverse physiological and developmental processes in humans including the development and function of melanocytes as well as immune cells **(Mansuri et al., 2016).**

Recently, miRNA-196-a2 gained a lot of attention. It has been reported to be deregulated in various cancer types and consequently, this up- or down-regulation may impact tumor malignancy or drug resistance according to the downstream target genes it affects. Bioinformatics analysis had shown that miR-196-a2 could target many genes enriched in cell cycle regulation, survival, and apoptosis **(Fawzy et al., 2017).**

However, little is known regarding the contribution of genetic variations in miRNAs to the development of vitiligo **(Huang et al., 2012)**. **Manga et al. (2006)**, hypothesized that SNPs in miR-196a-2 could potentially alter the regulation of the expression of the target TYRP1, an enzyme that may use an oxidative inducer as a substrate and promote ROS formation by catalyzing quinone production in melanocytes, leading to individual susceptibility to vitiligo.

In addition, **Cui et al. (2015)**, also demonstrated that miR-196a-2 targets Tyrp1gene, and the rs11614913 T/C change in miRNA196a-2 could down-regulate the cellular level of ROS and protect human melanocytes from apoptosis by suppressing the expression of Tyrp1.

This study aimed to investigate the association between a functional single nucleotide polymorphism (SNP) of rs 11614913 in microRNA 196a-2 and the serum tyrosinase level in vitiligo patients and evaluate the effect of miRNA 196a-2 polymorphism on the response to treatment in vitiligo patients. Also, the current study tried to explore the presence of microRNA 196a-2 polymorphism in first degree relatives of vitiligo patients as a predictor of susceptibility to develop the disease.

Vitiligo could be considered as a polygenetic disease; however, the genetic risk is not absolute. So, positive family history is one of the strong associations with vitiligo. **Mohammed et al. (2015)**, found that the range of family history in vitiligo varied from 6.25% up to 38% in some studies. And this is in agreement with the current study results that demonstrated that positive family history was 36%. And this percentage is higher than that was concluded by **Butt et al. (2015),** which was 22%. The patients were considered to have a family history if they had one or more first- to third-degree relatives with this condition.

Vitiligo is widely accepted to be an autoimmune disease and commonly associated with other autoimmune diseases. In this study, it was found that about 18% of the patients suffered also from other associated autoimmune diseases. This is comparable to that was found by **Cui et al. (2015)**, who mentioned that the incidence was 13%. However, this percentage was less than that mentioned by **Huang et al. (2012)**, as it was 26% in his study. This may be due to the exclusion of diabetes mellitus from the list of autoimmune disease in the current study. The exclusion of diabetes from the autoimmune diseases was due to the fact that the autoimmune mechanism plays a minor role in the pathogenesis of this disease especially type II, as the genetic, ethnic, environmental as well as nutritional factors play the main pathogenic roles **(Adeghate et al., 2006).**

VASI score was used together with the subjective degree of patient satisfaction to assess the degree of improvement after 4 months of treatment. The objective assessment after the course of the treatment showed that 75% of patients reported improvement. This percentage is considered high when it is compared with other studies, however, most of the patients record minimal degree of improvement in their VASI score. So, this percentage of improvement is not surprising when discovering that 40% of patients record minimal improvement.

So, the total percentage of overall subjective improvement was 70%. Only 28% out of them showed significant improvement, while the rest (42%) showed fair improvement. On the other hand, 30% of the treated patients were resistant to treatment either by no response (22%), or even by worsening of the condition despite of compliance to treatment (8%).

These results are comparable to that achieved by **Natta et al. (2003),** who achieved significant improvement (more than 50% repigmentation) in about 42% of their studied patients. And 40% significant improvement was also achieved by **Bhatnagar et al. (2007).**

Regarding the level of serum tyrosinase level, it was found that there was a highly significant relation between the higher levels of serum tyrosinase enzyme and the development of vitiligo. The mean levels were 89.05pg/ml and 80.4pg/ml in group 1 and 2 respectively. This finding is similar to that concluded by **Cui et al. (2015),** who reported 95pg/ml and 80pg/ml for the case and control group respectively.

On the other hand, the level of serum tyrosinase failed to show any statistically significant difference between group 2a and group 2b. And when the enzyme was re-measured after the end of the course of treatment, serum tyrosinse level showed a non significant reduction in response to treatment.

Serum tyrosinase level had no relation to gender. This is in agreement with **Cui et al. (2015).** He mentioned that the level is increasing with age, but the current study showed that there was no relation between the level of serum tyrosinase and the age of the patient.

This study revealed that there was a highly significant relation between the level of serum tyrosinase and the age of onset. As the higher levels of the enzyme were found in cases of early onset vitiligo. The same was reported by **Huang et al. (2012),** who also recommended the use of serum tyrosinase level as a predictive marker for the early-onset of vitiligo.

The serum Tyrosinase level could predict the response to treatment of patients with vitiligo. The patients with higher serum Tyrosinase levels tend to become more resistant to treatment and record less improvement in their VASI score.

T allele of miRNA 196a-2 polymorphism was found to have a significant relation with the development of vitiligo, as it was more prevalent in group 1. The same was stated by **Huang et al. (2012),** who also found that incidence of vitiligo development was high with TT genotype and low with CC genotype of the same gene.

Again, there was a highly significant relation between the genotype of miRNA 196a-2 and both the age of onset and the degree of improvement. **Jin et al. (2010)**, stated that genetic causes of vitiligo usually associated with earlier age of onset. This is in agreement with this study,

as it was demonstrated that the presence of TT genotype was associated with a high prevalence of early onset vitiligo.

Although the impact of miRNA 196-a2 polymorphism on the response of treatment in vitiligo patients was not investigated previously by any published article, the current study found that there is a great value for the genomic polymorphism on predicting the response to treatment. It was found that CC genotype showed a better response to treatment in comparison to TT genotype. So, the degree of improvement in both VASI score as well as patient satisfaction is significantly less in patient with TT polymorphism.

Although this study showed no statistically significant relation with the presence of associated autoimmune disease or with positive family history, the opposite was concluded by **Huang et al. (2012),** who found that CC genotype is associated with less association with other autoimmune disease and also less incidence of positive family history.

MiRNA-196a-2 C allele reduced the protein level of Tyr in PIG1 cells by inhibiting the expression of Tyrp1 **(Cui et al., 2015).** Also, the experimental data showed that up-regulation of miR-196a-2 expression decreased the protein levels of TYRP1, whereas inhibition of miR-196a-2 expression increased the protein levels **(Huang et al., 2013)**. These facts are completely in agreement with the current results. As it was found that CC genotype of miRNA 196a-2 polymorphism is associated with lower level of serum tyrosinase level (87.18 ±2.29) than TT genotype (90.4 ±1.89).

## CONCLUSION

The level of serum tyrosinase is higher in patients with vitiligo. And the levels of the enzyme were significantly higher in cases suffering from early onset vitiligo. On the other hand, the level of serum tyrosinase failed to show any statistically significant difference between the relative and control groups.

Patients with higher serum Tyrosinase level tended to be more resistant to treatment, and reported less degree of satisfaction. And the treatment course of vitiligo failed to record a significant reduction in the serum Tyrosinase levels.

However, the higher levels of serum Tyrosinase failed to record any significant relation with type or the severity of the condition. In addition, the serum Tyrosinase enzyme level was not affected by the age or the gender of the patient.

T allele of miRNA 196a-2 polymorphism was found to have a significant relation with the development of vitiligo. And presence of TT genotype not only predicts the development of the disease but also could predict the early age of onset and poor response to treatment.

On the other hand, miRNA-196a-2 C allele is associated with lower level of serum tyrosinase level than TT genotype, and less liability to develop vitiligo.

While the type of polymorphism was not related to neither the course of the disease, the type of vitiligo, the presence of positive family history nor to the associated autoimmune diseases.

Finally, it is concluded that miRNA 196a-2 polymorphism could be used as a predictive marker for the expected development of vitiligo. However its insignificant relation with the presence of positive family history suggests that vitiligo is a polygenetic disease in which other several genes are suspected to play a role in the etiopathogenesis of this disease.

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