ORIGINAL ARTICLE

Bali Medical Journal (*Bali MedJ*) 2022, Volume 11, Number 2: 587-590
P-ISSN.2089-1180, E-ISSN: 2302-2914

Correlation between Polymorphisms Interleukin-1 Receptor Antagonist (IL-1RA) And Melasma Severity: A Study of Javanese Female Population in Yogyakarta

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Received: 2022-05-04
Accepted: 2022-07-02
Published: 2022-07-11

ABSTRACT

Background: IL-1 is postulated as a cytokine that plays a fundamental role in the pathophysiology of several skin disorders or diseases. Melasma is a form of hyperpigmentation with a symmetrically distributed predilection for facial features. Due to its elusive pathogenesis, it is believed that the inflammatory factor plays a role in melasma. Increased or decreased IL-1RA concentrations may correlate with the severity of melasma. Therefore, this study aimed to examine the correlation between the IL-1RA (86bp VNTR) polymorphisms and the severity of melasma among a Javanese female population.

Methods: The study involved 79 individuals with melasma and used a cross-sectional design. The severity of melasma was assessed using the MASI (Melasma Area and Severity Index). The data were analyzed using a Chi-square test and Odds Ratio (OR). The genomic DNA from the patients' blood was examined and analyzed using the polymerase chain reaction (PCR) method.

Results: There was an increase in the frequency of IL-1Ra*1/1(410/410) genotype in melasma with a mild severity and of IL-1Ra*1/2(410/240) genotype in melasma with moderate severity (OR: 0.74, 95%CI: 0.26- 2.13, p: 0.42). The allele frequency of IL-1Ra*1/1 was 55.70% at a mild degree and 44.30% at a moderate degree (OR: 0.29-2.11, p: 0.65).

Conclusions: There was no correlation between IL-1Ra VNTR and the severity of melasma; however, it was found that the IL-1Ra*1/1(410/410) genotype and allele frequency tended to be higher in mild melasma, although it was statistically insignificant.

Keywords: IL-1Ra, Melasma, MASI, PCR, the severity of melasma.
Cite This Article: Suryaningsih, B.E., Sadewa, A.H., Wirohadidjojo, Y.W., Soebono, H. 2022. Correlation between Polymorphisms Interleukin-1 Receptor Antagonist (IL-1RA) And Melasma Severity: A Study of Javanese Female Population in Yogyakarta. *Bali Medical Journal* 11(2): 587-590. DOI: 10.15562/bmj.v11i2.3481

INTRODUCTION

Interleukin-1 (IL-1) is a proinflammatory cytokine encoded by two different genes, which are IL-1α and IL-1β. IL-1 is also postulated as a cytokine that plays a fundamental role in the pathophysiology of several skin disorders or diseases.[1,2] Alongside these two ligands, another family of IL-1, or the IL-1 receptor antagonist (IL-1RA), functions as a specific inhibitor of IL-1α and IL-1β. The structure of the IL-1RA variant is a 152 amino acid protein, which is the long arm of chromosome.[2]

Meanwhile, keratinocytes produce a significant amount of IL-1α, and in the epidermis, such quantity is critical for the biological activity of IL-1α. All IL-1 elements are found in the epidermis. Therefore IL-1 is believed to play a central or important role in physiological and pathological processes in the skin or play a fundamental role in various inflammatory reactions in the skin.[2-4] The variable number of tandem repeats (VNTR) is a form of polymorphisms in the IL-1RA gene associated with inflammation, autoimmunity, and tumors. The frequency of this polymorphism in the population depends on the geographical location and ethnicity from where an individual comes from.[5]

Melasma is a skin disorder in the form of hyperpigmentation with a symmetrically distributed predilection for the face. This disorder mostly occurs in women of their reproductive age and those from darker-skinned races or individuals with Fitzpatrick skin types III - V, although it can also occur in men.[6,7] Melasma often negatively impacts the sufferers' quality of life in terms of their social relationships in which they experience such feelings as embarrassment, low self-esteem, and dissatisfaction with their appearance. In some cases, a high melasma area and severity index (MASI) score can lead to suicidal intent.[8,9] Melasma is a skin disorder whose pathogenesis has not been fully elucidated, but it is believed to be multifactorial, including the inflammation factor.

This study aimed to examine the correlation between the polymorphisms

in the IL-1RA gene (86bp VNTR) and the severity of melasma among a Javanese female population.

METHODS

This was a cross-sectional study involving 79 female subjects with melasma. The patients were 18-60 years old Javanese women recruited at the Be Queen skincare clinic in Yogyakarta. All the subjects were interviewed with a standard questionnaire, including about their family history of melasma. The severity of melasma was measured using the Melasma Area and Severity Index (MASI). The MASI assessment measured three factors, including the Area of involvement (A), Darkness or Hyperpigmentation (D), and Homogeneity (H) based on a facial skin examination. The face was divided into four areas consisting of the Forehead (F) with a proportion of 30%, Right Malar Region (RM) with 30%, Left Malar Region (LM) with 30%, and Chin (C) with 10%. The four areas of involvement were scored from 0 to 6 (0 = no involvement, 1 = <10%, 2 = 10–29%, 3 = 30–49%, 4 = 50–69%, 5 = 70–89%, and 6 = 90–100%). The hyperpigmentation and homogeneity were assigned a scale from 0 to 4 (0 = absent, 1 = slight, 2 = mild, 3 = marked, and 4 = maximum). The final MASI score was the hyperpigmentation and homogeneity score multiplied by the facial area of involvement, resulting in a 0-48 score range.[10]

The exclusion criteria for this study were the use of hormonal contraceptives, pregnancy, pigmentation disorders other than melasma, and the use of whitening cream during the last two weeks before the study.[11] All the subjects were examined using the BombTech Skin Diagnosis A-ONE® (Korea) to identify the skin condition and area of melasma. Meanwhile, to measure the skin pigmentation and MASI score, the Mexameter® MX18 Courage-Khazaka (Germany) was used.

3 mL of venous blood was withdrawn from the subjects and stored in EDTA for DNA extraction with the Wizard® kit (Promega Corporation, Fitchburg, WI, USA). A polymerase chain reaction (PCR) was performed using the Platinum® PCR SuperMix kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) at the Biochemistry Laboratory, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia. The IL-1RA VNTR primers were from the research by Bid et al. (2004)[12] that included (F)5'-CTC AGC AAC ACT CCT AT-3' and (R) 5'-TCC TGG TCT GCA GGT AA-3' (Integrated DNA Technologies). The composition for PCR was 15 µl master mix from GoTaq Green Master (Promega) in a microcentrifuge tube, two µl of F+R primers, 11 µl of Nuclease-Free Water, and two µl of DNA template, all with a total volume of 30 µl. The tube was placed in a thermal cycler and the cycling program was started. The recommended parameters for the cycling condition consisted of initial denaturation at 95°C for 5 minutes, annealing at 95°C for 30 seconds, and elongation at 58°C for 30 seconds, at 72°C for 30 seconds, and at 72°C for 10 minutes (30 replications of denaturation – annealing – elongation). Electrophoresis was then conducted with 3% agarose (1st BASE produced by Genetic Science), and four µl of ethidium bromide (Promega) was added, followed by gel molding. The Carestream Gel Logic machine was used to examine the electrophoresis results.

This research has been approved by the Medical and Health Research Ethics Committee of the Faculty of Medicine, Universitas Gadjah Mada and Dr. Sardjito Hospital with approval letter No. KE/FK/462/EC/2016. All the participants had provided their written informed consent for this study. The study was conducted in accordance with the 1964 Declaration of Helsinki and its respective amendments and recommendations on 2015 ICH E6 (R1) Good Clinical Practice.

The data were analyzed in a chi-square test with $p < 0.05$ to determine the association between the VNTR of IL-1RA and the severity of melasma, and the Odds Ratio (OR) was calculated with 95% IC. The allele frequency and genotype frequency were estimated by calculating the expected value according to the formula $p2 + 2pq + q2 = 1$ of the Hardy-Weinberg Equation (HWE) with $p > 0.05$. The test resulted in an insignificant p-value, indicating that the frequency of the alleles in the population of Javanese women in Yogyakarta reached a state of equilibrium (Hardy Weinberg Equilibrium) from one generation to another of the sampling. The chi-square test was employed because the total number of subjects exceeded 40, and more than five observations in each cell were expected.

RESULTS

87 subjects were recruited, and 79 met the inclusion criteria. As many as eight (8) people were excluded because two of them (2) were diagnosed as being pregnant after a pregnancy test, two (2) were still using whitening cosmetics, three (3) were using hormonal contraceptives, and one (1) was having freckles. All of the subjects had skin type IV. There were 46 subjects with a family history of melasma from the parents (58.23%), while 33 people had no history of melasma in their family (41.77%). The severity of melasma was mild in 46 individuals (58.23%) and moderate in 33 subjects (41.77%).

Electrophoresis of PCR in 30% Agarose Gel

The electrophoresis of the IL-1RA gene VNTR in 3% agarose, which was observed

Table 1. Characteristics of the Research Subjects

Variable	N=79 (%)	P
MASI Score		
Mild	44 (55.69)	
Moderate	35 (44.31)	0.421
Age		
≤ 40 years	44 (55.69)	
> 40 years	35 (44.31)	0.059
Family History		
With melasma	46 (58.23)	
Without melasma	33 (41.77)	< 0.001

Significance: $p < 0.05$

with a Carestream Gel Logic reader, showed a band in each replication of the IL-1RA gene sequence (86bp). The electrophoresis results of IL-1RA gene VNTR were based on the markers used as a reference (control).

Regarding the genotype, we found IL-1RA*1/2 and IL-1RA*1/1 in the research subjects. The IL-1RA*1/1 genotype was found at a higher frequency in mild melasma than in moderate melasma (44.30%:32.91%). Meanwhile, the IL-1RA*1/2 genotype had the same frequency in mild and moderate severity (11.39%:11.39%) (Table 2). The statistical analysis showed that the difference in proportion was not statistically significant.

Regarding the allele frequency, the statistical analysis also showed a non-significant result. However, the allele frequency can not be calculated because there were only IL-1RA*1/1 and IL-1RA*1/2 genotypes (The IL-1RA*2/2 was absent) (Table 3).

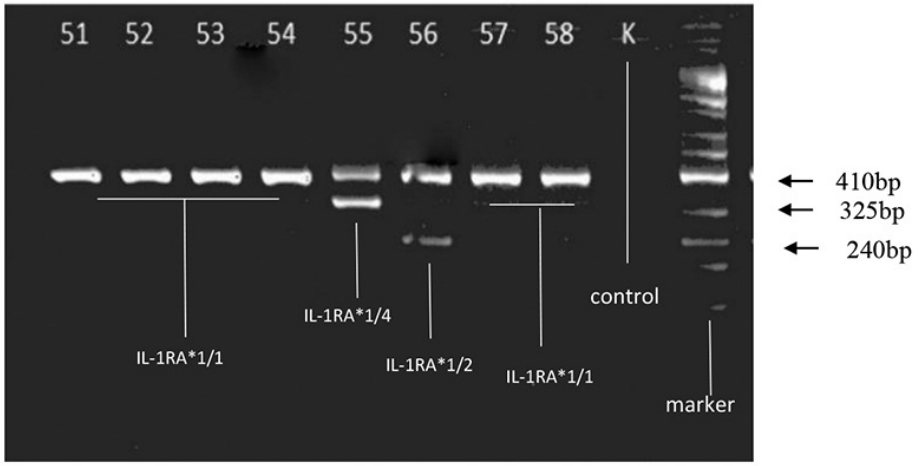

Figure 1. Results of electrophoresis of the IL-1RA gene VNTR. Subjects 51-54 and 57-58 were IL1-RA* 1/1, showing only one band at 410bp (410/410). Subject 56 was IL-1RA* 1/2 with two bands, 410bp and 240bp (410/240). Subject 55 was IL-1RA* 1/4 with two bands of 410bp and 325bp (410/325). K = control

Table 2. Genotype Frequency of IL-1RA VNTR by the Severity of Melasma

Genotype	Moderate	Mild	OR (CI 95%)	P
IL-1RA*1/2	9 (11.39)	9 (11.39)	Ref	
IL-1RA*1/1	26 (32.91)	35 (44.30)	0.74 (0.26-2.13)	0.42

Significance: $p < 0.05$

Table 3. Allele Frequency of IL-1RA VNTR by the Severity of Melasma

Allele	Moderate	Mild	OR (CI 95%)	P
IL-1RA*1/2	9	9	Ref	
IL-1RA*1/1	70 (44.30)	88 (55.70)	0.79 (0.29-2.11)	0.65

Significance: $p < 0.05$

DISCUSSION

Melasma is a disorder commonly occurring in reproductive-aged women and is even more frequent in individuals with skin types III-V of the Fitzpatrick classification. Melasma can affect the face and other parts of the body that are exposed to the sun.[6,13] Melasma often interferes with the quality of life of the affected individuals, thus causing a lack of confidence. In some cases, a high MASI score often triggers suicidal intent. The pathogenesis of melasma remains inadequately elucidated. Genetic factors presumably associated with such disorder include exposure to ultraviolet light, hormones, and inflammatory processes.[14,15] The genetic factors and UV exposure are believed to be adequate risk factors for melasma. The Val92 Met genotype is among the genotypes that play a role in the occurrence of melasma, thus presenting evidence of existing genetic factors that play a role in melasma incidence.[16-18]

The IL-1RA gene is located at 2q14-21 adjacent to the IL-1α and IL-1β genes. The variable number of tandem repeats (VNTR) polymorphism within intron 2 of the IL-1RA gene is caused by a repeat in every 86 bp of the gene. The frequency of IL-1RA VNTR in each individual varies depending on the ethnicity and geographic location of the population. There are five recognized allele variants of the IL-1RA VNTR gene: (1) IL-1RA*1 with four repeats measuring 410bp, (2) IL-1RA*2 with two repeats measuring 240bp, (3) IL-1RA*3 with five repeats measuring 500bp, (4) IL-1RA*4 with three repeats measuring 325bp, and (5) IL-1RA*5 with six repeats measuring 595bp.[5,12,19] The highest frequency in healthy populations is that of homozygotes with four repeats of IL-1RA*1 compared to the two repeats of IL-1RA*2.[12] In this study, the frequency of IL-1RA*1/1 was higher in the group with mild melasma than in the group with moderate melasma, while no severe melasma was found.

IL-1α is produced in large amounts by keratinocytes, and the overexpression of IL-1α in the epidermis can lead to an inflammatory skin reaction, scale formation, and hair loss.[3] Inflamed skin will experience an increased secretion of IL-1α, and a high concentration of IL-1RA is needed to block or stop IL-1α signal transduction to prevent further damage. The concentration ratio needed to stop an inflammation is between 10:1 to 500:1, depending on the concentration of the IL-1 receptor and the target cell. The ratio of IL-1RA: IL-1α in normal skin is approximately 120:1.[4]

IL-1RA is a competitive inhibitor of the bioactive IL-1. The concentration of IL-1RA usually rises during inflammation. This high concentration will prevent the process of acute inflammation from turning into chronic inflammation and will not damage healthy cells. IL-1RA is likely to play a role in the severity of a disease.[20,21]

The results of a previous study indicate that IL-1RA VNTR can minimize damage to the cells and stop ongoing inflammation.[3] In this present study,

the IL-1RA VNTR in the homozygous IL1RA*1/1 was found more frequently in the subjects with mild MASI, while that in the heterozygous IL-1RA*1/2 was more common in those with moderate MASI (44.30%:32.91%). The IL-1RA*1/1 genotype was a protective factor for the moderate severity of melasma (OR: 0.74, 95% CI: 0.26-2.13), but it was insignificant ($p > 0.05$). This study indicated that the more repeats of the IL-1RA gene, the more adequate the protection it provides against the severity of melasma. The mechanism for inflammation in melasma and the role of IL-1 in inducing melanogenesis were likely to underlie the role of IL-1RA in this study as a protective factor against melasma.

CONCLUSION

The study of the correlation between the severity of melasma and the VNTR of IL-1RA was the first study conducted in a Javanese population. In this study, mild severity of melasma or mild MASI was higher in the IL-1RA VNTR of homozygous IL1RA*1/1 compared to in that of heterozygous IL-1RA*1/2 genotype (44.30%:32.91%). The IL-1RA*1/1 genotype was a protective factor for the occurrence of moderate severity of melasma (OR: 0.74, 95% CI: 0.26-2.13). To conclude, more repeats of the IL-1RA gene will provide adequate protection against the severity of melasma.

ACKNOWLEDGMENTS

We appreciate the participation of all the subjects who have agreed to be involved in this research.

CONFLICT OF INTEREST

The authors declare no conflicts of interest in this work.

ETHICS APPROVAL

This research has been approved by the Medical and Health Research Ethics Committee of the Faculty of Medicine, Universitas Gadjah Mada and Dr. Sardjito Hospital with approval letter No. KE/FK/462/EC/2016

FUNDING

No third-party funding was involved in this research

AUTHOR CONTRIBUTION

All authors contributed equally in this research

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