ORIGINAL ARTICLE

Bali Dermatology and Venereology Journal (*BDV*) 2019, Volume 2, Number 1, 12-16
P-ISSN.2089-1180, E-ISSN.2302-2914

Published by DiscoverSys

The positive correlation between serum malondialdehyde levels with vitiligo severity and activity

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ABSTRACT

Background: There are several theories that suggest melanocyte death in vitiligo, one example is oxidative stress theory. Oxidative stress primarily manifested by lipid peroxidation ultimately produce malondialdehyde. Malondialdehyde is a stable marker to assess an oxidative stress event. The correlation between serum malondialdehyde levels and vitiligo severity and activity remained controversial in previous studies.

Objective: This study aimed to verify whether or not the serum MDA levels are positively correlated with vitiligo severity and activity.

Methods: This study was an analytical cross-sectional study. which involved 64 subjects with vitiligo and 20 subjects without vitiligo. Serum MDA levels were measured to mark an oxidative stress event, whereas the severity and activity of vitiligo were clinically assessed with vitiligo area severity index (VASI) and vitiligo disease activity (VIDA) scoring system.

Results: Total 64 subjects with vitiligo and 20 subjects without vitiligo participated in this study. Serum MDA mean levels of vitiligo subjects were significantly higher compared to subjects without vitiligo ($p<0.05$). Serum MDA levels had a strong positive correlation with VASI score ($r=0.761$; $p<0.01$). The strong positive correlation found between serum MDA levels with vitiligo activity assessed by VASI score in vitiligo subjects ($r=0.609$; $p<0.01$), and high serum MDA levels increased the risk for developing vitiligo (PR=7.62; 95% CI: 2.49-23.30; $p<0.01$). Serum MDA levels influenced vitiligo as much as 10.1%, meanwhile the remaining 89.9% were influenced by other variables apart from high serum MDA levels out of this study ($R^2=0.101$; $p<0.05$).

Conclusion: Serum MDA levels were positively correlated with vitiligo severity and activity, and high serum MDA levels increased the risk of developing vitiligo.

Keywords: malondialdehyde, vitiligo, VASI, VIDA

Cite this Article: Praharsini, I.G.A.A., Wiraguna, A.A.G.P., Batan, P.N.W.2019. The positive correlation between serum malondialdehyde levels with vitiligo severity and activity. *Bali Dermatology and Venereology Journal* 2(1): 12-16. DOI: 10.15562/bdv.v2i1.17

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Received: 2019-12-07
Accepted: 2019-12-24
Published: 2019-08-01

INTRODUCTION

Vitiligo is the most commonly found acquired pigmentation disorder due to melanocyte death. 50% of vitiligo cases were found before the age of 20, but vitiligo can be found in every age group.[1,2]

The aetiology of vitiligo is not fully understood yet, but some theories suggested that oxidative stress may cause melanocyte death which will ultimately lead to vitiligo.[3] Melanocyte became the subject of oxidative stress due to reactive oxygen species (ROS) exposure during the melanin synthesis. Oxidative stress may alter melanocyte homeostasis and lead to melanocyte death.[4,5]

ROS is able to interact with polyunsaturated fatty acid (PUFA), initiating lipid peroxidation which produces malondialdehyde (MDA) and trans-4-hydroxy-2-nonenal (4-NHE).[6] Lipid peroxidation is the main manifestation and first parameter of oxidative stress used by researches to prove the involvement of ROS to cell injury. MDA is the main metabolic product used to evaluate lipid peroxidation, which can be used for a reliable oxidative stress biomarker.[7]

Serum MDA measurement can be used to predict oxidative stress levels.[9-11] Several studies showed controversial results of the relation between MDA levels and vitiligo, not to mention studies regarding correlations between serum MDA and vitiligo severity remain limited. The abovementioned statements thus lead to the conception of the ideas where this study aimed to comprehend any correlations between serum MDA levels and vitiligo severity index.

MATERIALS AND METHODS

This is an analytical cross-sectional study conducted in Dermatovenerology Outpatient Clinic of Sanglah General Hospital Denpasar on the period of January to March 2018. Serum MDA levels vitiligo area severity index (VASI) and vitiligo activity degree measured by vitiligo disease activity (VIDA). MDA mean differences between subjects

with and without vitiligo, and also whether or not high levels of serum MDA will increase the risk for vitiligo. Anamnesis, physical examination, and the measurements of VASI and VIDA scores were done in Dermatovenerology Outpatient Clinic of Sanglah General Hospital Denpasar, meanwhile serum MDA level measurements were done in Clinical Pathology Laboratory of Sanglah General Hospital Denpasar. Research samples were classified into subjects with and without vitiligo, which are taken from the reachable population using a consecutive sampling method. Patients with vitiligo which went to Dermatovenerology Outpatient Clinic of Sanglah General Hospital Denpasar, Indonesian, both men or women aged 5 to 75 years old with the good general condition and were willingly participating into this study confirmed by signing an informed consent were included in this study. Subjects which have undergone topical, systemic, or phototherapy vitiligo treatment minimum of 2 weeks prior to this study, in pregnancy, in possession of chronic systemic disease such as chronic renal failure, coronary heart disease, rheumatoid arthritis, systemic lupus erythematosus, diabetes mellitus, bronchial asthma, malignancy, hepatic cirrhosis, HIV infection, along with other chronic skin inflammation such as psoriasis and atopic dermatitis, history of smoking, alcohol consumption, and systemic anti-inflammatory treatment in the last 4 weeks, and consumption of antioxidants such as vitamins A, C, E, selenium, and zinc in the last 4 weeks are excluded from this study. The severity of vitiligo is classified into mild (VASI score < 5), moderate (VASI score 5 – 10), and severe (VASI score >10) which were assessed through physical examination. The activity of vitiligo is measured by VIDA score by analysing the progressivity and stability of the vitiligo. VIDA score ranges from the minimum of -1 and maximum of +4 which were assessed through anamnesis. MDA level measurements are done using a spectrophotometer with the Competitive-ELISA method. Statistical tests were used using Statistical Package for Social Sciences (SPSS) software version 21.0.

Table 1 **Research subjects characteristics**

Characteristics	Vitiligo (n= 64)	Non vitiligo (n=20)
Sex		
Male	36 (56.25%)	4 (20%)
Female	28 (43.75%)	16 (80%)
Age (years)		
5 – 15	7 (10.94%)	0 (0%)
16 – 25	6 (9.37%)	0 (0%)
26 – 35	8 (12.50%)	18 (90%)
36 – 45	7 (10.94%)	2 (10%)
46 – 55	20 (31.25%)	0 (0%)
56 – 65	11 (17.19%)	0 (0%)
66 – 75	5 (7.81%)	0 (0%)
Onset		
Early-onset	22 (34.38%)	N/A
Late-onset	42 (65.62%)	N/A
Vitiligo type		
Non-segmental	47 (73.44%)	N/A
Segmental	5 (7.81%)	N/A
Unclassifiable/ Undetermined	12 (18.75%)	N/A
Family history		
Present	9 (14.06%)	N/A
None	55 (85.94%)	N/A
Serum MDA mean levels	295.48 ± 225.60	136.01 ± 113.53

Table 2 **Normality tests results**

No	Variable	p-value
1	Serum MDA levels of subjects with vitiligo	0.200•*
2	Serum MDA levels of subjects without vitiligo	0.000º
3	VASI score	0.005•
4	VIDA score	0.002•

*Normal distribution if p>0.05, •Kolmogorov Smirnov test used, º Shapiro-Wilk test used

RESULTS

This study involved 84 subjects which fulfilled both inclusion and exclusion criteria, which are grouped into 64 subjects with vitiligo and 20 subjects without vitiligo. The characteristics of the subjects are listed in Table 1.

Kolmogorov-Smirnov normality test was used to assess the normalities of serum MDA levels of subjects with vitiligo, vitiligo severity assessed by VASI score, and vitiligo activity assessed by VIDA score. On the other hand, the Shapiro-Wilk normality test was used to assess the normality of serum MDA levels of subjects without vitiligo due to the number of subjects being less than 50 subjects. The results of normality tests can be seen in Table 2.

Mann-Whitney test results showed that serum MDA mean levels of subjects with vitiligo were significantly different compared to subjects without vitiligo with p-value <0.05 (Table 3).

Due to the abnormal data distribution, Spearman's rho correlation test was used in this study to comprehend the correlation of serum MDA

Table 3 Serum MDA mean levels of differences between subjects with and without vitiligo

Subject group	n	MDA mean levels ± SD	Mean rank	p-value
With vitiligo	64	295.48 ± 225.60	48.13	0.000*
Without vitiligo	20	136.01 ± 113.53	24.50	

*p-value is significant <0.05; SD= standard deviation

Table 4 Correlation between serum MDA level and vitiligo severity based on VASI score

		VASI score
Serum MDA levels	r	0.761
	p	0.000*
	n	64

*p-value is significant <0.05

Table 5 Correlation between serum MDA levels and vitiligo activity based on VIDA score

		Skor VIDA
Serum MDA levels	r	0.609
	p	0.000*
	n	64

*p-value is significant <0.05

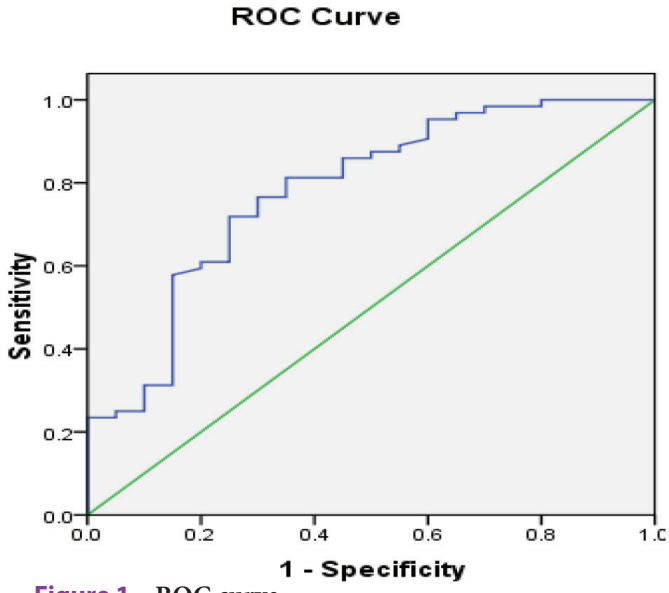

Figure 1 ROC curve

Table 6 Bivariate analysis of serum MDA levels toward vitiligo

		Group		PR	95% CI	p-value
		Vitiligo	Non-Vitiligo			
MDA	High	49 (76.6%)	6 (30.0%)	7.62	2.49-23.30	0.000*
	Normal	15 (23.4%)	14 (70.0%)			

*p-value is significant <0.05

levels with vitiligo severity based on VASI score. Results showed that there was a strong positive correlation between serum MDA levels and vitiligo severity based on VASI score (r=0.761; p<0.01) as shown in Table 4.

Spearman's rho correlation test was used to comprehend the correlation between serum MDA levels and vitiligo activity based on VIDA score due to the abnormally distributed data. Results showed that there was a strong positive correlation between serum MDA levels and vitiligo activity based on VIDA score (r=0.609; p<0.01), which can be seen in Table 5.

ROC curve showed that the cut-off point of serum MDA level was 135.075 ng/mL with the sensitivity of 76.6% and specificity of 70.0% (Figure 1)

Serum MDA level was considered high if it exceeds 135.075 ng/mL, and was considered normal if less than or equals to 135.075 ng/mL. Chi-square test was used to comprehend the relationship of serum MDA level and vitiligo, as shown in Table 6.

Table 6 showed that there was a relationship between serum MDA levels and vitiligo. High serum MDA levels increased the risk for developing vitiligo for 7.62 times (RP 7.62; 95%CI 2.49-23.30; p < 0.01) compared with normal serum MDA levels.

DISCUSSION

Vitiligo is mostly found in the young and middle stage of life, but it can also occur in any stages of life. In this study, the youngest subject which had vitiligo was 1 year old and the oldest was 72 years old, with the mean onset age of 33.97 ± 12.23 years old. Based on the onset, vitiligo is classified into early-onset vitiligo (onset <30 years old) and late-onset vitiligo (onset >30 years old).[11,12] In this study, subjects with early-onset vitiligo attributed for 34.38% while the remaining 65.62% had late-onset vitiligo. A study by Lazzeri et al. (2016) had the similar results in which out of 191 patients with vitiligo, late-onset vitiligo was more commonly found (64.40%) compared to early-onset vitiligo (35.60%).[13]

Segmental vitiligo is the most common type found in this study (73.44 %). Lazzeri et al. (2016) shared similar results in which out of 188 patients with vitiligo, the non-segmental type was more commonly found (94.68%), followed by segmental type (4.26%) and unclassifiable vitiligo (1.06%).[13] Wang et al. (2013) conducted a similar study in China with the results which showed that non-segmental vitiligo is the most common vitiligo found in the community.[14] Another study by Sun et al. (2006) and Mchepange et al. (2010) also showed similar results.[15,16]

In this study, 14.06% of the subjects had a

Published by DiscoverSys | *BDV* 2019; 2(1): 12-16 | doi: 10.15562/bdv.v2i1.17

family history of vitiligo, with the remaining 85.94% had none. Familial researches which focus on the involvement of genetical factors in vitiligo vulnerability showed that vitiligo is a complex multifactorial disorder which can be passed down polygenically. Results of this study is supported by results of a study by Sun et al. (2006) which reported that out of 815 patients with vitiligo, 128 patients (15.7%) had a family history of vitiligo; 59.6% of which had history from the first degree of the family and 55.2% from the second degree of the family.[15] Another study by Mchepange et al. (2010) showed that 5-20% of patients with vitiligo in China had a family history of vitiligo.[16]

Mann-Whitney test was done, and results showed that there was a significant difference in serum MDA mean levels between subjects with and without vitiligo ($p < 0.05$). This result is supported by a study conducted by Pande and Gupta (2017) which involved 40 subjects, in which subjects with vitiligo had an increased serum MDA mean levels of 3.39 nmol/L.[17] Singh et al. (2016) studied 60 subjects with vitiligo and 60 subjects without vitiligo and concluded that serum MDA mean levels were notably higher in patients with vitiligo compared to patients without vitiligo, with a mean difference of 3.72 nmol/mL.[10] Dammak et al. (2006) measured the serum of 36 patients with vitiligo and 40 control patients to comprehend the redox status marker of MDA serum levels. Results showed that serum MDA levels on subjects with vitiligo were higher compared to subjects without vitiligo.[18]

The elevation of serum MDA levels on subjects with vitiligo compared to subjects without vitiligo resembled an elevation of lipid peroxidation which can be seen as an oxidative stress main manifestation on subjects with vitiligo. High serum MDA levels on subjects with vitiligo also meant that there was a higher oxidative stress level on subjects with vitiligo compared to subjects without vitiligo.[10,19]

Results of this study showed that there was a strong positive correlation ($r = 0.761$; $p < 0.01$) between serum MDA levels and vitiligo severity calculated based on VASI score. There were no studies which correlated serum MDA levels and vitiligo severity based on VASI score before, but one study correlated plasma MDA levels with vitiligo severity based on VASI score. This study shared the same results with a study conducted by Widayati et al. (2017) in which stated that there was a significant positive correlation ($r = 0.94$; $p < 0.001$) between plasma MDA levels and vitiligo severity based on VASI score. The study measured the plasma MDA levels with the results of 72.59 ng/mL for patients with mild vitiligo, 132.32 ng/mL for moderate vitiligo, and 472.68 ng/mL for severe vitiligo.[20]

This study also showed that there was a strong positive correlation ($r = 0.609$; $p < 0.01$) between serum MDA levels and vitiligo activity calculated based on VIDA score, as shown in Table 5. The correlation level was stronger than expected as stated in the study hypothesis ($r = 0.4$ It can be concluded that the higher the serum MDA level is, the higher the VIDA score which reflects the vitiligo activity gets. Mitra et al. (2017) conducted a similar study in India using 51 patients with vitiligo and 54 controls. Results showed that MDA mean levels of subjects with active vitiligo were significantly higher compared to healthy control (3.48 ± 0.17 compared to 2.87 ± 0.07 mM; $p < 0.05$).[21]

This study found out that high serum MDA level increased the risk of vitiligo for 7.62 times (RP 7.62; 95% CI = 2.49-23.30; $p < 0.01$) compared to subjects with normal serum MDA levels. A study conducted by Haider et al. (2010) also showed that serum MDA level was significantly higher in subjects with vitiligo compared to the control group ($p < 0.05$).[19]

This study has several limitations, one of them is the cross-sectional design used in this study. This design is considered weak to determine the causal-effect relationship of the study, thus further studies are needed to ensure that high serum MDA level is a risk factor for vitiligo. Additional studies are also needed to evaluate serum MDA and its relations to both enzymatic and non-enzymatic antioxidants to comprehend the instability of oxidants and antioxidants on oxidative stress more, which ultimately leads to melanocyte death in vitiligo.

CONCLUSION

Subjects with vitiligo had notably higher serum MDA level compared to subjects without vitiligo. Serum MDA level of patients with vitiligo had a strong positive correlation with vitiligo severity based on VASI score. There was a strong positive correlation between serum MDA levels of subjects with vitiligo and vitiligo activity based on VIDA score. High serum MDA levels increased the risk of vitiligo for 7.62 times compared to normal serum MDA levels. Serum MDA levels affect vitiligo as much as 10.1%, while the rest 89.9% are affected by other variables except high serum MDA level apart from this study.

ETHICAL CLEARANCE

This study was ethically approved by the Research Ethical Commission of Medical Faculty of Universitas Udayana/Sanglah General Hospital with the ethical code: 2302/UN.14.2/KEP/2017

CONFLICT OF INTEREST

Authors stated there is no conflict of interest in this study.

FUNDING

The study preparation, laboratory examination, analysis, and publication was funded by authors own funding.

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