

Smoking as A Risk Factor of Low Superoxide Dismutase (SOD) Levels and Early Aging in Women Aged 20 to 35 Years

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ABSTRACT

Background: Heavy smokers have four times more facial wrinkles than non-smokers. Superoxide dismutase (SOD) levels are an indicator of premature aging in blood and tissues. The premature aging process can take place more quickly at a relatively younger age. The purpose of this study was to determine smoking and low levels of superoxide dismutase (SOD) as risk factors for premature aging in women aged 20-35 years. **Method:** Using a case-control study, this study determined smoking and low levels of superoxide dismutase (SOD) as risk factors for premature aging in women aged 20-35. Subjective clinical observation of wrinkles with the smoker's face criteria (Daniel 1971) and objective use A-One-Facial Analyzer tool. Analyze using SPSS 26. **Result:** The smoking increased the risk of premature aging by 16 times (OR 16; 95% CI= 4,2-60,7); $p < 0,001$ and passive smoker 36 times (OR 36; IK95% 7,6-168,9; $p < 0,001$). Low SOD levels (< 2.93 U/ml) increased the risk of premature aging by 9.7 times (OR 9.7; 95%CI =3.2-29.1); $p < 0.001$ and levels of superoxide dismutase (SOD) of smokers were lower than non-smokers with a median (IQR) [min-max] of smokers 1,5 (1,6) [0,4-9,7]U/ml while non-smokers were 7,5 (6,3) [0,1-14,2]U/ml and $p < 0.001$. The results of the multivariate analysis showed that smoking and low SOD as risk factors for premature aging in women aged 20-35 years with smoking (AOR 18; 95%CI=5,5-66,8; $p < 0,001$) and low SOD (AOR 10,2; 95%CI=1,5-67,1; $p < 0,001$). **Conclusion:** That smoking and low levels of superoxide dismutase (SOD) as risk factors for premature aging in women aged 20-35 years.

Keywords: premature aging; smoker's face; smoking; superoxide dismutase

INTRODUCTION

Premature aging due to exposure to external factors such as sunlight, cigarette smoke, and pollution in general causes an increase in reactive oxygen species (ROS) in the body, ROS itself can cause protein oxidation and damage to Deoxyribonucleic acid (DNA), resulting in a decrease in collagen synthesis and increase collagen degradation, both of which cause premature aging of the skin [1].

One indicator of premature aging is the superoxide dismutase (SOD) level in the blood and tissues. SOD is an enzyme that neutralizes various free radicals or ROS, which play an important role in the pathophysiology of premature aging. Under conditions of oxidative stress, SOD levels were found to decrease. Therefore, a decrease in SOD levels can also indicate premature aging [2].

A WHO survey in 2008 shows that a third of the world's population, especially adults, are smokers. According to Risesdas in 2018, the definition of active smokers is the habit of smoking every day, or sometimes in the past month, passive smoking is being near people who are smoking in one room; the proportion of active smokers in Indonesia is 24.3, in Bali 18.9 with average smoking of 12 cigarettes per day, and the highest proportion is at the age of 20-55 years, while men have a proportion of 47.3% and women 12.2%, while for passive smokers, the proportion is 27% for men and 34% for women [3].

Ernster et al. Smokers have an odds ratio (OR) of 3.1 for premature aging compared to non-smokers[4]. As we know, the negative impact caused by cigarettes is not only experienced by active smokers, but passive smokers also obtain these effects, so the risk of organ damage in passive smokers is the same as in active smokers [5].

Smoking can increase ROS in the body; the more ROS, the body's natural antioxidants, such as SOD, will decrease and cause premature aging. The case-control study found that serum SOD was lower in smokers compared to the control group. The more number of cigarettes per day (> 10 cigarettes) consumed, the lower the serum SOD, the longer the duration of smoking (> 10 years) the lower the levels. SOD. This study was conducted to determine the relationship between smoking habits and SOD levels in blood plasma as an indicator of premature aging.

METHOD

This is an analytic observational study with a case-control design conducted at the Dermatology and Venereology Polyclinic, Prof. Dr. IGNG Ngoerah Hospital Denpasar. Inclusion criteria: (1) Subjects with and without signs of premature aging who visited the Dermatology and Venereology Polyclinic, Prof. Dr. IGNG Ngoerah Hospital, Denpasar period June 2022- August 2022;(2) Indonesian, aged between 20 to 35 years with female gender; (3) Willing to carry out peripheral blood sampling procedures,

willing to be involved in research and signing informed consent; (4) General condition is good. Exclusion criteria: (1) Subjects were suffering from chronic inflammatory skin diseases such as psoriasis and atopic dermatitis; (2) Have had plastic surgery or facial reconstruction; (3) Have had a facial resurfacing procedure; (3) Have or are currently doing skin treatments such as botulinum toxin injections, fillers and the use of topical antiaging creams on the face with effects still visible on screening day; (4) The subject is a pregnant woman who is known from the anamnesis; (5) The subject is a woman who is breastfeeding her child; (6) Subjects were consuming antioxidants such as vitamins A, C, E, selenium and zinc and topical antioxidants such as vitamins A, C, and E in the last four weeks; (7) Subjects used antiaging creams containing retinoids, hydroxy acids or alpha hydroxy acids (AHAs).

Samples were recruited by consecutive sampling. Data collection process and performed anamnesis, physical examination, smoker's face score examination (Daniel, 1971) subjective assessment of premature aging. Supportive examination using the A One Smart Skin Analyzer tool to objectively determine premature facial skin aging on the subject and take 3 mL of venous blood to examine plasma SOD levels at the Clinical Pathology Laboratory of the Integrated Biomedical Laboratory of FK Udayana. The data collected will be examined, processed, and analyzed using statistical tests with the Statistical Package for Social Sciences (SPSS) 24.0 program.

RESULTS

This study involved 78 subjects who met the inclusion and exclusion criteria. The results of the characteristics of the subjects in this study are presented in Table 1.

TABLE 1: Characteristics of research data.

Characteristics	Cases (n=39) (%)	Control (n=39) (%)
Age (Years) mean±SD	28.2±5.3	29.1±5.9
Work		
Private sector employees	23 (59)	21 (53.8)
Self-employed	3 (7.6)	3 (7.6)
Student	3 (7.6)	8 (20.5)
Government employees	4 (10,2)	4 (10,2)
Cleaning Service	6 (15.4)	3 (7.6)
Ethnic group		
Bali	25 (64.1)	28 (71.8)
Java	14 (35.9)	11 (28.2)
Length of smoking/exposure to cigarette smoke (years) mean±SD	3.3±1.2	2.4±0.6
Prolonged exposure to the sun		
< 2 hours/day	20 (51.3)	13 (33.3)
2-<3 hours/day	13 (33.3)	24 (61.5)
3-5 hour/day	6 (15.4)	2 (5,1)
Duration of cigarette exposure/day		
2-<3 hours/day	16 (41)	3 (7.6)
3-5 hours/day	9 (23.1)	6 (15.4)
> 5 hours/day	9 (23.1)	-
Smoking habit		
Never smoked	5 (12.8)	30 (76.9)
Passive smoker	18 (46.2)	3 (7.6)
Active and Passive Smokers	16 (41.1)	6 (15.4)
Smoking intensity		
Light Smoker	1 (2.6)	1 (2.6)
Medium Smoker	6 (15.4)	4 (10,3)
Heavy Smoker	9 (23.1)	1 (2.6)
Smoker's Face Premature Aging Value (Daniell, 1971)		
1	0	39 (100)
2	32 (82)	0
3	7 (18)	0
Use of antioxidants	0	0
Use of sunscreen/face care	0	0
Mean±SD A-One- Facial Analyzer	4.7±0.6	3.4±0.3
Mean±SD SOD U/ml	0.8±0.4	7.2±4.6

The A-One-Analyser Facial data was obtained with a range of 3.0 to 5.9, with a median of 4. Furthermore, the statistical method of the Receiver Operating Characteristic (ROC) procedure was carried out and assessed the Area Under the Curve (AUC) to determine the threshold for the presence of wrinkles. The ROC curve (Figure 1) shows that the A-One Facial Analyzer has excellent diagnostics because the curve is above the 50% line.

The AUC value obtained from the ROC method was 98.6% (95% CI 0.965–1,000, $p < 0.001$). Statistically, the AUC value shows excellent diagnostic power. The results of ROC coordinates show cut-off value used in this study is 3.95, which has a sensitivity of 97.4% and a specificity of 94.9%.

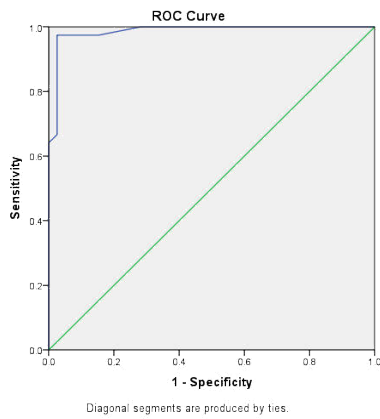


FIGURE 1: The results of the ROC A-One- Analyzer Facial procedure for the presence of wrinkles with an AUC value of 98.6%.

In this study, none of the subjects used antioxidants because all subjects felt there was no need to take antioxidant drugs and none of the subjects used sunscreen/face care because most of the subjects in the study were in a closed room that was not exposed to direct sunlight, so they were not exposed to the sun. There is also a need the use sunscreen. In this study, to find out how much smoking can cause premature aging in women aged 20-35 years, an analysis was carried out with bivariate analysis in Table 2.

TABLE 2: Smokers a risk factor for premature aging in women aged 20-35 years compared to not active smoking.

Smoke	Group		OR	95% CI	p- value
	Case n=39 (%)	Control n=39 (%)			
Never smoked	5 (12.8)	30 (76.9)			
Passive smoker	18 (46.2)	3 (7.6)	36	7.6-168.9	<0.001
Active smoker	16 (41.1)	6 (15.4)	16	4.2-60.7	<0.001

Significant p<0.05; a: test results Square

In this study, there were differences in the mean levels of superoxide dismutase (SOD) in smokers and non-smokers because the distribution was not expected, soused a non-

parametric method, namely the Mann-Whitney test, which obtained a significance value (p) of <0.001 (<0.05) with a 95% confidence interval (Table 3).

TABLE 3: Differences in superoxide dismutase (SOD) levels between smokers and non-smokers in women aged 20-35 years.

Variable	Group		p-value
	Smoke n=22	Never Smoked n=35	
SOD level (U/ml)	1.5 (1.6)	7.5 (6.3)	<0.001 ^a
Median (IQR)	[0.4-9.7]	[0.1-14.2]	
min-max			

Significant p<0.05; a: test results Mann-Whitney

DISCUSSION

The result of age between the two groups was not much different, namely in the case group with a mean±SB of 28.2±5.3 years and in the control group with 29.1±5.9 years. This mean age is to research conducted by Sanusi, Sawitri, and Putri (2020), who found that the age with the highest percentage of premature aging was 20-30 years, with 128 subjects (96.2%) in the 20-25 year age range and eight subjects (6.02%) in the 26-30 year age range due to smoking.[6]. Subjects aged 20-35 years, according to Pangkahila's criteria for premature aging, are included in the subclinical category because free radicals from exposure to cigarette smoke cause premature wrinkles. The premature aging process can occur more quickly at a relatively younger age. Premature aging can occur intrinsically and extrinsically; premature aging occurs intrinsically due to biological phenomena, namely, increasing age and the influence of hormones. Extrinsic factors can be influenced by sun exposure and smoking, where smoking tends to be more dominant in accelerating premature aging[7].

80.7%, the relationship between age and wrinkle in female smokers was found to be significant with a regression coefficient of -1.15 and p < 0.01 this indicates that young people who smoke are 1.15 times more likely to have wrinkles than non-smokers[8]. In 148 women aged 30-39 years, severe wrinkles were found to be 2.9 times higher than in non-smoking women (95% KI 1.6-5.5) (P<0.01)[4].

In most jobs, private employees work indoors with less than 2 hours of sun exposure, so this exposure does not affect premature aging or research results. One hundred twenty minutes of sun exposure from 10:00 to 16:00 a day can cause skin wrinkles and signs of photoaging [9].

Heavy smokers smoked more than 15 cigarettes daily in this study, the most in the case group, nine people (23.1%). This result is from the Gavali study (2019), where smoking at least 20-80 cigarettes will increase the risk of decreasing SOD. A cross-sectional study of 100 patients found that SOD levels were low in patients with a habit of smoking 5 to 10 cigarettes a day. Another cross-sectional study that included 60 smokers with a consumption of 100 packs of cigarettes per year also found low levels of SOD [10].

This result is slightly different from that obtained by Lange and Schnohr Severe Wrinkle at the age of 30-39 years by

Several case-control studies with 50 smokers as the case group and 50 non-smokers as the control group found lower serum SOD in smokers compared to the control group, the more cigarettes per day (> 10 cigarettes) consumed, the lower the serum SOD, the lower the serum SOD. The longer the duration of smoking (> 10 years), the lower the SOD level. The duration of smoking/exposure to cigarette smoke was found in the more extended case group, with mean±SD 3.3±1.2 years. In the control group, 2.4±0.6 years were sufficient to reduce SOD levels and cause premature aging.

The study showed that smoking increases premature aging by 16 times compared to never smoking. Daniell also reported that smoking harms the skin, characterized by smokers' faces, a hallmark of smoking. Dupati and Helfrich (2009) also stated that heavy smokers had four times more facial wrinkles than non-smokers. Research in Korea found that smoking can cause the risk of wrinkles on the face as much as 11 times compared to people who do not smoke; even a study in Japan found the risk of developing wrinkles as much as 22 times in people who smoke compared to non-smokers in men [12].

The results are also from the research conducted by Maedasari et al. (2015). They found a significant relationship between smoking and premature aging in the form of wrinkles in the Area around the eyes ($p < 0.05$). Women who smoke have a ten times higher chance of experiencing premature aging in the form of wrinkles in the eyes than non-smokers (PR 10, 95% CI 1.41-70.99, $P < 0.05$). Master's research (1995) also states that women have a 3.1 times risk of premature aging due to smoking compared to men, who are only 2.3 times. Okada's research (2013) also stated that female smokers are more sensitive to the risk of premature aging than men after inhaling ten packs per year. In men, wrinkles appear after inhaling 20 packs per year.

The eye area of smokers is more prominent than in other locations wrinkles because smokers will more often experience the blink reflex to protect the eyes from the presence of cigarette smoke which is irritating to the eyes. When this blinking occurs, it is played by the orbicularis muscle surrounding the eye, resulting in repeated eye muscle movements due to contraction and relaxation of the orbicularis muscle. Repetitive muscle movement is one of the external factors that cause premature aging (Masnec, 2010; Drakaki, 2014; Nall, 2013).

This study also found that passive smoking has a 36 times risk of premature aging compared to never smoking. This is by a study conducted in China on 874 subjects aged 20-40 years who detected cases of passive smoking with wrinkles and found a significant correlation with $r = 0.657$; $p < 0.001$, which means that there is a strong correlation between passive smoking and aging with a risk of 7.7 times compared to non-smokers. This study also states that exposure to cigarette smoke with a duration of 120 minutes per day in a room with a diameter of 2.5 square meters for at least six months will increase the incidence of premature aging by 66.7%. [14]. Li et al. (2014) also conducted [15] the same research on 857 women aged 30-40 years; 74% of women experienced premature aging when exposed to cigarette smoke in their homes, with a risk of 3.6 times, $p = < 0.001$.

In research conducted by Muizzuddin et al. (1997), comparing the effects of smoking on patients who are active smokers, passive smokers, and never smoked with the effects on the skin in 100 people in New York aged 35-45 years, it was found that the risk of active smokers more than 50 packs per year experiencing wrinkles was 4.7 times.

With $p = 0.026$, not much different from the results in passive smokers with exposure to cigarettes regularly every day in the home environment and right at work are known to have a risk of 4.2 times with $p < 0.001$. The analysis results also showed no significant difference between passive and active smokers on skin with wrinkles with $p > 0.05$. This is also similar to research conducted by Donald (1987) which states that passive smokers also have almost the same risk of wrinkles, which is 3.1 times.

In this study, it was found that between active and passive smokers, the risk of wrinkles was higher in passive smokers; this is by the study Ortiz, and Grando (2012) stated that passive smoking exposure of at least 1,000 hours per year increased the risk by 3.2 times greater than that of active smokers of 20 packs per year. One of the factors that cause passive smoking to have a higher risk is because the intensity of exposure to passive smoking is higher in terms of duration of exposure in the home environment, work environment, and the assumption that they are only exposed to regular cigarette exposure. Hence, they feel safe even though the risk of exposure to the skin has the same risk against aging.

This study proved that smoking is a risk factor for premature aging. The effect of active smoking and passive smoking is riskier for those not exposed to cigarette smoke to experience premature aging. This result has significant meaning in educating women to prevent aging. Early childhood has an important meaning in premature aging programs and education for smokers not only to stop smoking but also to avoid exposure to cigarette smoke, in preventing premature aging from patients and family support to stop smoking or not smoking in the house or at home. In a designated smoking area. It is also important for the implementation of smoke-free areas, that all indoor areas should be smoke free to prevent exposure to passive smoker.

Several studies are not by the results of this study, namely in a study conducted by Schnohr that women who smoke do not eat significantly wrinkles at the age of 20-39 years; this is because there is no exclusion from the use of facial treatments. In Allen's study, it was found in 650 subjects, 79% of white people and 21% of black people aged 20-40 years who smoked a pack per day, it was found that there was no difference in wrinkling scores between smokers and non-smokers on black skin, skin, and skin. In white, it was found that Daniel's mean score did increase in smokers, but after the analysis test, there was also no difference.

Difference research wrinkles in twins, the results of 2 different studies were found, in a study conducted by Doshi et al. (2007) on 52 identical white twins who smoked 52 packs per year found that smokers had more wrinkles than non-smokers, while the study of Haruko et al. (2013) conducted on 79 subjects of identical twin pairs after five years of observation, it turns out that there is no difference in facial structure and wrinkles in smokers and non-smokers.

This study's results were different because the research subjects came from black or white areas. At the same time, the researchers examined skin with Fitzpatrick type 4, and the number of daily cigarette exposures was also different. Some of the causes that make women who smoke do not develop wrinkles are due to the consumption of vegetables, fruits, and spices that are rich in antioxidants and the use of natural treatments such as frequent use of fruits, flowers, or herbal plants as masks or use to be mixed when cleaning. Face, and the use of cosmetics containing sunscreen.

Low SOD levels (<2.93 U/ml) increased the risk of premature aging by 9.7 times (OR 9.7; 95% CI = 3.2-29.1); $p < 0.001$) compared with high SOD levels (≥ 2.93 U/ml). The skin has defense mechanisms to fight oxidative stress, including cell repair involving DNA Repair Enzymes. In the epidermis, SOD is the most important antioxidant in protecting the epidermis. Suppose the level of SOD is low in plasma. In that case, it will indicate that the number of free radicals (pro-oxidants) is greater than the number of endogenous defense substances (antioxidants), which indicates oxidative stress [1]. In research conducted by Djawad (2021), giving SOD 250 IU for 60 days twice a day can increase total antioxidant status (TAS), reduce Transepidermal Water Loss (TEWL) and sebum concentration due to photoaging in 25 subjects, Fitzpatrick skin type 4, 14 men and 11 women with age 20 subjects (30-40 years) and five subjects (25-29 years).

Superoxide dismutase has been tested in several studies since the 1990s, in vivo, and in vitro, using either topically or orally. Research on the topical use of SOD was conducted by Benyahia et al., 1996; Filipe et al., 1997; Chen et al., 2016. In the Benyahia study carried out on 42 patients with cutaneous fibrosis after radiotherapy and given topical SOD containing 10 mg of PEG-SOD (3.6X10⁴) 2 times a day for three months, the results after a biopsy of the skin tissue were found that SOD topically was able to form collagen in the skin. The irradiated dermis layer reactivates cellular function in fibroblasts and epidermal cells.

In Filipe's study, two volunteers used SOD gel with a concentration of 1% two times a day for ten days with UV exposure 4 J/cm² longwave ultraviolet lamp, model B 100 2 hours per day, with biopsy examination of the skin tissue before and after the procedure, it was found that SOD was able to protect the skin layer from damage caused by UV.

In Chen's study, ten healthy men with Fitzpatrick skin type II used TAT-SOD 300 units/cm² hours before UVB rays for three months with a UVB exposure of 2 hours per day found that TAT-SOD was able to avoid UVB-induced skin breakdown in men.

In this study, it was found that the smoking group had a lower median SOD (IQR) [min-max] than non-smokers, 1.5 (1.6) [0.4-9.7] ng/L while those who never smoked 7.5 (6.3) [0.1-14.2]. The results of this study were also obtained in research conducted by Gavali et al. (2019), with the results that smokers had a mean \pm SD of 2.50 \pm 0.45 U/mL compared to non-smokers with a mean of 3.01 \pm 0.23 U/mL. Cigarette use of 16.02 \pm 3.2 packs per day with a total of 1 pack of 12 cigarettes is known to have a serum SOD level of 2.50 \pm 0.45 U/mL with moderate correlation results (Pearson correlation $r = -0.52$) and $p = 0.0032$, which means that the amount of smoking will make SOD levels decrease [10]. Nandi and Chatterjee (1988) state that the normal value of serum SOD is 2.93-3.71 U/mL. A study by van der Vaart (2004) stated that the free radicals from smoking that cause oxidative stress are at risk of increasing the decrease of SOD 5-10 times.

In the study, 21 female smokers found SOD levels were lower than 21 non-smokers, with the results 1867.99 \pm 395.4 (U/mg.prot) vs. 2256.89 \pm 458.2 (U/mg.prot); $p < 0.001$ (Ozguner et al., 2005). In the study of 14 women aged 25-29 who smoked, it was found that SOD levels were lower than 11 healthy women without smoking, namely 1.545 \pm 0.174 U/mL vs. 1.187 \pm 0.169 U/mL ($p < 0.05$) [18].

CONCLUSION

Based on the research and discussion results, it can be concluded that smoking and low levels of SOD (< 2.93 U/ml) are risk factors for premature aging.

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