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Assessment of plasma malondialdehyde levels among free-diver fishermen in southeast Maluku district: exploring influencing factors on oxidative stress

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ABSTRACT

Background: *Indonesia, with its expansive territorial waters, hosts numerous fishing communities residing on various islands. Many of these communities rely on diving activities, predominantly free diving without standardized safety equipment. This practice poses risks, including the potential for hypoxia-induced oxidative stress, which plays a role in disease pathogenesis. This study aimed to investigate the levels of malondialdehyde (MDA) in freediving fishermen and explore potential influencing factors.*

Materials and methods: *The research involved 30 freediving fishermen, aged 20–60, who engaged in diving at least twice weekly over the last 3 months. Blood plasma MDA levels were assessed using the Will method.*

Results: *Results revealed a median age of 40.5 years (range: 20–59), a body mass index of 23.1 ± 2.8, and a mean blood pressure of 132/85 mmHg. A significant portion of the subjects exhibited smoking habits (90%) and alcohol consumption (76.7%). The median MDA level among subjects was measured at 0.42 nmol/mL (range: 0.34–0.70). However, no discernible relationship was found between smoking habits, alcohol consumption, and MDA level categories, as determined by the Fisher exact test ($p > 0.05$).*

Conclusions: *While these findings shed light on the MDA levels in freediving fishermen, further research is warranted to explore additional factors that may influence these levels. This comprehensive understanding is crucial for addressing the health risks associated with free diving practices in this unique population.*

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Keywords: malondialdehyde (MDA), Will's, smoking, alcohol, hypoxia

INTRODUCTION

Indonesia's vast archipelagic expanse, encompassing over 6.4 million km^2 of marine territory with a coastline stretching over 108,000 km, defines a nation teeming with marine resources [1]. Coastal and island-dwelling communities, constituting a substantial portion of Indonesia's population, heavily rely on fishing as their primary livelihood. Despite the abundant potential, many Indonesian fishing communities persist in utilizing traditional methods, notably free diving, an age-old practice where breath-holding skills are passed down through generations. Unfortunately, this tradition often lacks formal training and proper equipment, posing health and safety risks to practitioners due to the absence of standard diving gear [2, 3].

Freediving, characterized by single-breath plunges without breathing apparatus, presents environmental chal-

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lenges as divers navigate increasing hydrostatic pressure and encounter substantial variations in oxygen saturation during apnoea. Techniques like hyperventilation and glossopharyngeal insufflation are employed to enhance freediver performance, yet these methods carry their own set of risks, including barotrauma and cerebral hypoperfusion. Beyond 20 meters, freediving exposes individuals to significant health hazards, including the dangers of hypoxic syncope and shallow water blackout [4, 5]. This risk is particularly evident among traditional fishermen, such as those in X Village, Southeast Maluku Regency, who continue to grapple with limited access to health and safety resources.

The health risks associated with freediving extend beyond physical challenges, triggering inflammatory responses and impacting immune function, especially among traditional fishermen who engage in this practice without standardized safety equipment. The intricate interplay of hypoxia, oxidative stress, and the activation of the NF-κB signalling pathway underscores the complexity of health issues faced by these vulnerable communities [6–8]. Addressing these concerns requires a holistic approach encompassing improved safety measures, enhanced healthcare access, and community-wide awareness initiatives to safeguard the overall well-being of traditional fishermen in Indonesia [4].

While research on malondialdehyde (MDA) levels in free divers remains limited, a notable study by Kozakiewicz et al. [9] has shed light on potential connections between free diving and oxidative stress. The research found a significant increase in MDA concentration in erythrocytes after exposure to hyperbaric conditions, indicating heightened oxidative stress among participants. This aligns with the observations made by Pilz et al. [10], who noted a substantial rise in total MDA levels in healthy volunteers following hypoxic treatment — a condition akin to the challenges faced by free divers. Despite these insights, direct studies examining specific MDA levels in free divers are currently lacking, leaving a gap in our understanding of the oxidative stress implications unique to this group.

This research aims to comprehensively examine MDA levels in freediving fishermen, while concurrently investigating the various factors influencing these levels. The overarching objective is to generate a nuanced overview that can serve as a foundation for educational initiatives aimed at improving the safety and health of the community, particularly among fishermen. The study aims to fill existing knowledge gaps and provide practical insights that empower the community to make informed decisions regarding safety practices during freediving activities.

MATERIALS AND METHODS

SUBJECTS

This study adopts a cross-sectional design, serving as a preliminary investigation presented in a descriptive format and involving 30 subjects. Thirty freediving fishermen from Village X in the Southeast Maluku district, meeting the inclusion criteria of being adult men aged 20–60 years and traditional fishermen engaging in diving activities without equipment at least 2 times a week in the last 3 months, volunteered to participate as research subjects. The exclusion criteria for this study were applied to individuals with a history of fever or infectious diseases. Ethical approval for the research has been duly granted by the FKUI-RSCM ethics committee under No. KET-1060/UN2.F1/ETIK/PPM.00.02/2023. Additionally, the study holds an official research location permit in the Southeast Maluku district, sanctioned by the National Unity and Politics Agency of the Southeast Maluku Regency Government, with research recommendation No. 070/151/SIP/BKBP/2023.

Comprehensive data collection was undertaken for each subject, encompassing demographic information such as age, body weight, height, and vital signs, including blood pressure (BP), pulse, and respiratory frequency. Additionally, structured interviews were conducted to gather specific details, such as the frequency of diving per week, depth of diving, any history of disturbances or complaints following diving activities, and information about smoking habits and alcohol consumption history. This multifaceted approach to data collection aims to provide a thorough understanding of both the participants' baseline characteristics and their freediving-related experiences and habits.

BLOOD SAMPLING

Blood sampling involved extracting five millilitres of whole blood from the subject's cubital vein using a 5 cc syringe. The collected blood was then carefully deposited into a vacutainer tube containing EDTA anticoagulant. Subsequently, the blood underwent separation through centrifugation at 3500 rpm for 10 minutes, ensuring the isolation of plasma from blood cells. The separated plasma was then transferred into a new microtube. To preserve the integrity of the samples, all plasma specimens were appropriately stored in either a –20°C or –80°C freezer until the time of measurement for the research parameters.

MDA MEASUREMENT

In this study, MDA concentration measurements were conducted using the Will method. The procedure involved six standard solution tubes, each containing distilled water and MDA standards, with a total volume of 400 µL. The blank tube consisted of 400 µL of distilled water. Furthermore, the test tube contained 100 µL of plasma and 300 µL of distilled water. To ensure precision, each tube was duplicated, and 200 µL of 20% trichloroacetic acid (Merck) (TCA) was added to each. The solution in each tube underwent

homogenization using a vortex, followed by centrifugation for 10 minutes at a speed of 5000 rpm. The resulting supernatant was carefully transferred into a microtube. Subsequently, 400 µL of 0.67% thiobarbituric acid was introduced into each microtube, followed by homogenization using a vortex. Each microtube was then incubated in a water bath for 10 minutes at a temperature ranging from 95–100°C. Post-incubation, the tubes were cooled with water, and the process concluded with absorbance readings at a wavelength of 530 nm using a spectrophotometer. The MDA concentration for each sample was determined using the formula derived from the MDA standard curve [11].

DATA ANALYSIS

The descriptive data derived from the outcomes of this study are presented in two formats based on the distribution of the data. For normally distributed data, the mean and standard deviation (± SD) are utilized, providing a central tendency measure along with a measure of variability. Conversely, for non-normally distributed data, the median along with the minimum and maximum values (median [min–max]) is employed to accurately depict the data's central position and range. Furthermore, categorical data is summarized using a frequency table, offering a clear presentation of the count and corresponding percentages for each category.

RESULTS

In this preliminary study, 30 freediving fishermen meeting the inclusion criteria were recruited, and their characteristics are detailed in Table 1. The median age of the subjects was 40.5 years, ranging from 20 to 59 years, and they exhibited a mean body mass index of 23.1 ± 2.8. The average systolic BP was recorded at 132 ± 11 mmHg, with a mean diastolic BP of 85 ± 9.5 mmHg. The subjects displayed an average pulse rate of 75.7 ± 10 beats per minute, while the median respiratory frequency was 20 times per minute, ranging from 16 to 24. These baseline characteristics provide an initial data of the study population and form the foundation for further analysis and interpretation.

Table 1. Characteristics of freediving fishermen

Characteristics	Mean ± SD/median (min–max)
Age [years]	40.5 (20–59)
Body mass index [kg/m^2]	23.1 ± 2.8
Systole BP [mmHg]	132 ± 11
Diastole BP [mmHg]	85 ± 9.5
Pulse [times/min]	75.7 ± 10
Respiration rate [times/min]	20 (16–24)

BP — blood pressure; min–max — minimum-maximum; SD — standard deviation

Table 2. Smoking and alcohol consumption habits of subjects

Characteristic (n = 30)	Number (persons)	Percentage (%)
Smoking habits		
Yes	27	90
Frequency:		
Rare	7	23.3
Often	20	66.7
No	3	10
Alcohol consumption habits		
Yes	23	76.7
Frequency:		
Rare	14	46.7
Often	9	30
No	7	23.3

Table 3. Malondialdehyde (MDA) level of freediving fishermen's plasma

Parameter (n = 30)	Median/number
MDA [nmol/mL]	0.42 (0.34–0.70)
Group:	
Low (persons)	15 (50%)
High (persons)	15 (50%)

Table 2 presents the history of smoking habits and alcohol consumption among freediving fishing subjects. A significant majority, comprising 90% of the subjects, reported being active smokers. Among these, 66.7% indicated a frequent smoking habit. However, detailed information regarding the number of cigarettes smoked per day and the age at which subjects initiated smoking was not obtained, precluding the calculation of the Brickman index. Notably, only 10% of the subjects were non-smokers, providing insights into the prevalent smoking behaviour within this study.

The consumption of alcohol among freediving fishing subjects is detailed in Table 2. The majority, constituting 76.7% of the subjects, reported alcohol consumption, with 46.7% indicating rare intake. Conversely, 23.3% of the subjects reported never consuming alcohol. It's noteworthy that specific data regarding the quantity of alcohol consumed by individual subjects was not collected, limiting a more detailed analysis of alcohol consumption patterns within this study.

The outcomes of plasma MDA measurements for the subjects are outlined in Table 3, with a median MDA level of 0.42 nmol/mL (range: 0.34–0.70). The table categorizes subjects based on MDA levels in relation to the median. Subjects with MDA levels lower than the median are classified as the low MDA group, whereas those with equal to or higher than the median are placed in the high MDA group. Notably, 50% of the subjects exhibited MDA levels surpassing the median, placing them in the high MDA group category, while the remaining 50% were classified in the low MDA

Table 4. The proportion of the malondialdehyde (MDA) category based on the habits of freediving fishing subjects

	MDA level	
	Low	High
Smoking habits		
Yes	14	13
No	1	2
Alcohol consumption		
Yes	12	11
No	3	4

Fisher exact test, $p > 0.05$

Table 5. The proportion of the malondialdehyde (MDA) category based on habits frequency of the freediving fishing subjects

	MDA level	
	Low	High
Smoking frequency		
High	9	11
Low	5	2
No	1	2
Alcohol frequency		
High	5	4
Low	7	7
No	3	5

Fisher exact test, $p > 0.05$

group category. This categorization facilitates a clear understanding of the distribution of MDA levels within the study.

Among smoking subjects as presented on Table 4, 51.9% exhibited low MDA levels, while 48.1% displayed elevated MDA levels. In contrast, within the non-smoking group, 33.3% of participants showed low MDA levels, with the remaining 66.7% presenting high MDA levels. Examining alcohol consumption history, 52.2% of those who consumed alcohol had low MDA levels, whereas 47.8% exhibited elevated MDA levels. Conversely, among non-alcohol consumers, 42.9% had low MDA levels, while the remaining 57.1% showcased high MDA levels.

According to the data in Table 5, among subjects with high smoking frequency, 55% demonstrated high MDA levels, while the remaining 45% exhibited low MDA levels. Conversely, in the low smoking frequency group, 71.4% had low MDA levels, and the remaining 28.6% displayed high MDA levels. In the group with high-frequency alcohol consumption, 55.6% presented low MDA levels, with the remaining 44.4% showing high MDA levels. On the other hand, in the group of subjects with low-frequency alcohol consumption, 50% had low MDA levels, while the rest exhibited high MDA levels.

DISCUSSION

In this preliminary study, we enlisted 30 participants who satisfied the specified criteria. Analysis of the demographic data related to the subjects reveals that free diving fishermen predominantly fall within the productive age bracket, spanning from 20 to 59 years. This observation aligns with findings from Ahmad et al. [12], who examined risk factors for decompression in divers in the Selayar district. They noted that a significant portion (44%) of their subjects fell within the age range of 26–45 years, with 35.2% falling in the younger bracket of 12–25 years, and the remaining participants falling into the older age category beyond 45 years. Similarly, Duke et al. [13], in their exploration of the impact of diving depth, diving time, and anaemia on decompression, reported an average age of 38 years for fishermen, with the majority falling within the range of 26–50 years. Consequently, our study appears to parallel both investigations in terms of the age distribution among the subjects.

Nutrition emerges as a pivotal consideration for divers, with specific recommendations of 3500 kcal for male competition divers and 2650 kcal for their female counterparts. Divers are advised to prioritize nutrient-dense foods, ample fluids, and essential supplements such as iron and calcium. However, a notable concern arises when divers excessively focus on achieving an ideal body weight and composition, potentially leading to inadequate energy availability and heightened health risks [14]. The nutritional status of the subjects under scrutiny predominantly placed them in the overweight category, with an average body mass index of 23.1. Unfortunately, our study lacks further exploration into the subjects' dietary intake and body composition. Consequently, the root cause of their overweight condition remains elusive, hindering the formulation of targeted educational interventions. Existing studies on United States Navy divers have uncovered patterns of excessive sodium and cholesterol consumption coupled with low fibre intake [15]. Intriguingly, there is a lack of research specifically examining the nutritional status of freediving fishermen. This underscores the need for future investigations tailored to this unique population, shedding light on their dietary habits and potential health implications.

The optimal BP is defined as a systolic BP of less than 120 mmHg and a diastolic BP of less than 80 mmHg. The normal category are individuals with a systolic BP of 120–129 mmHg and a diastolic BP of 80–84 mmHg. Those with a systolic BP of 130–139 mmHg and a diastolic BP of 85–89 mmHg are classified in the high normal category. The category of individuals with hypertension encompasses those with a systolic BP above 140 mmHg and/or a diastolic BP above 90 mmHg [16]. In this study, the average systolic BP recorded was 132 mmHg, and the diastolic

BP was 85 mmHg, placing it in the high normal category. It is recommended that individuals falling into this group initiate lifestyle change interventions. If cardiovascular disease is confirmed, additional medication may be considered as part of the management strategy [16]. This underscores the importance of proactive measures for individuals with BP levels in the high normal range to mitigate potential cardiovascular risks.

In this study, a predominant majority of subjects exhibited smoking habits (90%) and alcohol consumption (76.7%). This aligns closely with findings from Ahmad et al. [12], whose research on diving subjects revealed that 98% were smokers and 94.9% indulged in alcohol consumption. Similarly, Embuai et al. [17] reported high prevalence among diving fishermen in the Ambon area, with 91.30% having smoking habits and 82.6% engaging in alcohol consumption. Notably, smoking among diving fishermen was associated with a sixfold increase in the risk of decompression compared to non-smokers, according to existing research. Additionally, alcohol consumption was linked to a twofold increase in the risk of decompression compared to individuals who abstained from alcohol [12].

Malondialdehyde, the end product of the lipid peroxidation chain reaction, serves as a useful marker for assessing oxidative stress [11]. Studies on trained free divers indicated that repetitive static apnoea, performed 1, 3, and 5 times, did not yield significant changes in MDA concentrations. The baseline MDA concentration before static apnoea stood at 13.2 µmol/L, with the final concentration after 5 rounds of static apnoea reaching 14.2 µmol/L. There was a significant increase in superoxide dismutase and glutathione peroxidase activity after 5 instances of apnoea compared to the baseline. Concurrently, there was a decrease in catalase activity [18]. In our conducted research, we observed the median plasma MDA level among subjects in our study was 0.42 nmol/mL, ranging from 0.34 nmol/mL to 0.70 nmol/mL. In the statistical analyses conducted using the Fisher exact test, no significant relationship was identified between smoking and alcohol drinking habits and the categories of MDA levels. Similarly, the frequency of both smoking and alcohol consumption did not exhibit a discernible correlation with the MDA level categories. These results suggest that, based on the statistical testing performed, there is no apparent association between smoking, alcohol consumption, and the observed categories of MDA levels in the studied population. However, while our research contributes to the understanding of oxidative stress markers, there is a gap in the literature concerning MDA levels specifically in freediving fishermen. Future investigations focusing on MDA levels in this population could provide valuable insights into the oxidative stress dynamics associated with their unique diving activities.

In summary, our study revealed that freediving fishermen subjects were primarily in their productive age, exhibited normal high BP, and had a body mass index falling within the overweight category. A significant portion of the subjects were smokers and alcohol consumers. Examination of MDA levels showed a median of 0.42 nmol/mL, with no discernible relationship observed between smoking habits, alcohol consumption, and the categories of MDA levels.

It is essential to note that further studies are warranted, particularly those comparing MDA levels among freediving fishermen and non-diving controls. These future investigations should encompass various parameters of oxidative stress and antioxidants, aiming to provide a more comprehensive understanding of the health, safety, and security conditions within this unique population. Such endeavours are crucial for advancing our knowledge and fostering targeted interventions that promote the well-being of freediving fishermen.

ARTICLE INFORMATION AND DECLARATIONS

Data availability statements: Each author's contribution to this study has been thoroughly documented in the author contribution section. For any additional inquiries or further clarification may direct to the corresponding author.

Ethics statement: This study has received approval from the local ethics committee no. KET-1060/UN2.F1/ETIK/PPM.00.02/2023 and research permit from the local government No. 070/151/SIP/BKBP/2023.

Author contribution: S.S.: methodology, project administration, writing, F.V.I: investigation, data curation, E.A.: methodology, validation, N.S.H.: supervision, data curation, F.C.I.: conceptualization, funding acquisition, writing.

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Conflict of interest: All authors declare that there is no conflict of interest in this study.

Supplementary material: Not available.

REFERENCES

1. Susilo AFI, Harsono G, Wirasatya A. Pengaruh IOD (INDIAN OCEAN DIPOLE) terhadap variabilitas distribusi suhu permukaan laut dan klorofil-A pada tahun 2019 di perairan Meulaboh, kepulauan SInabang, Provinsi Aceh. Jurnal Hidrologi Indonesia. 2022; 4(2): 1–13.
2. Sukbar S, Dupai L, Munandar S. Relation Between Diving Activities With Vital Lung Capacity of Fishermen in Rural Torobulu District Laeya South Konawe 2016. Fak Kesehat Masy Univ Halu Oleo. 2016: 1–9.
3. Yuliana B, Mahmud NU, Sumiaty S. Faktor yang Berhubungan dengan Kejadian Penyakit Dekompresi pada Nelayan Penyelam

Tradisional di Pulau Barrang Lompo. Window Public Health J. 2021; 2(4): 648–656, doi: 10.33096/woph.v2i4.219.
4. Kusnanto K, Wabula LaR, Purwanto B, et al. Safety behaviour and healthy diving: a qualitative study in the traditional diverse fishermen. Int Marit Health. 2020; 71(1): 56–61, doi: 10.5603/IMH.2020.0012, indexed in Pubmed: 32212149.
5. Patrician A, Dujić Ž, Spajić B, et al. Breath-Hold diving - the physiology of diving deep and returning. Front Physiol. 2021; 12: 639377, doi: 10.3389/fphys.2021.639377, indexed in Pubmed: 34093221.
6. Farías JG, Herrera EA, Carrasco-Pozo C, et al. Pharmacological models and approaches for pathophysiological conditions associated with hypoxia and oxidative stress. Pharmacol Ther. 2016; 158: 1–23, doi: 10.1016/j.pharmthera.2015.11.006, indexed in Pubmed: 26617218.
7. Mrakic-Sposta S, Vezzoli A, D'Alessandro F, et al. Change in oxidative stress biomarkers during 30 days in saturation dive: a pilot study. Int J Environ Res Public Health. 2020; 17(19), doi: 10.3390/ijerph17197118, indexed in Pubmed: 32998440.
8. Suzuki K, Tominaga T, Ruhee RT, et al. Characterization and modulation of systemic inflammatory response to exhaustive exercise in relation to oxidative stress. Antioxidants (Basel). 2020; 9(5), doi: 10.3390/antiox9050401, indexed in Pubmed: 32397304.
9. Kozakiewicz M, Kędziora-Kornatowska K, Kaczerska D, et al. Influence of exposure in hyperbaric chambers on selected parameters of oxidative stress in professional divers. Undersea Hyperb Med. 2018; 45(1): 49–54, doi: 10.22462/01.02.2018.7.
10. Pilz J, Meineke I, Gleiter CH. Measurement of free and bound malondialdehyde in plasma by high-performance liquid chromatography as the 2,4-dinitrophenylhydrazine derivative. J Chromatogr B Biomed Sci Appl. 2000; 742(2): 315–325, doi: 10.1016/s0378-4347(00)00174-2, indexed in Pubmed: 10901136.
11. Hardiany N, Sucitra S, Paramita R. Profile of malondialdehyde (MDA) and catalase specific activity in plasma of elderly woman. Health Science J Indonesia. 2020; 10(2): 132–136, doi: 10.22435/hsji.v12i2.2239.
12. Ahmad A, Gobel FA, Mahmud NU. Faktor Risiko Terjadinya Dekompresi pada Penyelam di Kabupaten Kepulauan Selayar. J Muslim Community Health. 2023; 4(4): 23–33, doi: 10.52103/jmch.v4i4.1313.
13. Duke HI, Widyastuti SR, Hadisaputro S, et al. Effect of the depth of diving, duration of diving, anemia on decompression sickness in traditional diver. J Kesehat Masy Indones. 2017; 12(2): 12–18.
14. Benardot D, Zimmermann W, Cox GR, et al. Nutritional recommendations for divers. Int J Sport Nutr Exerc Metab. 2014; 24(4): 392–403, doi: 10.1123/ijsnem.2014-0012, indexed in Pubmed: 24936780.
15. Singh A, Deuster PA, Day BA, et al. Nutritional status of land-based U.S. Navy divers. Undersea Biomed Res. 1988; 15(2): 135–145, indexed in Pubmed: 3363752.
16. Lukito AA, Harmeiwaty E, Hustrini NM. Konsensus penatalaksanaan hipertensi. Perhimpunan Dokter Hipertensi Indonesian Society of Hipertension, Jakarta 2019: 96.
17. Embuai Y, Denny H, Setyaningsih Y. Analisis Faktor Individu, Pekerjaan dan Perilaku K3 pada Kejadian Penyakit Dekompresi pada Nelayan Penyelam Tradisional di Ambon. Jurnal Penelitian Kesehatan "SUARA FORIKES" (Journal of Health Research "Forikes Voice"). 2019; 11(1): 6, doi: 10.33846/sf11102.
18. Bulmer AC, Coombes JS, Sharman JE, et al. Effects of maximal static apnea on antioxidant defenses in trained free divers. Med Sci Sports Exerc. 2008; 40(7): 1307–1313, doi: 10.1249/MSS.0b013e31816a7188, indexed in Pubmed: 18580412.