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Effects of Diving Toward Lung Function, Levelss of Malondialdehyde (MDA) and Leukotrien-B_4(LTB_4) in Serum of Indonesian Navy Divers.

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ABSTRACT

Diving causes lung function changes is still need to be proven. Oxidative stress and decompression sickness caused by diving result in injury and inflammation of airway. Inflammatory mediators such as MDA and LTB_4 play role in it. The purposes of this study are to prove the effects of diving toward the decreasing of lung function, to know the changing levelss of MDA and LTB_4 and to prove the correlation among the decreasing of lung function, MDA and LTB_4 levelss in the serum of Indonesian Navy divers. Quasi-experimental, 40 samples consists of 20 divers who served in Indonesian Navy diving course Surabaya and 20 non-divers of Indonesian Navy who served in Academy of Indonesian Navy Surabaya. On the first month, both divers and non-divers had lung function examination and measurement of MDA and LTB_4 serum levelss as the baseline. Divers group who had dived 30 times (for 6 months) underwent re-examination of lung function, serum levelss of MDA and LTB_4. Lung function examination using spirobank II, while levelss of MDA and LTB_4 using ELISA method. There is significantly decreased of lung function; FVC ($p<0.001$), FEV_1 ($p=0.043$), PEF ($p<0.001$), FEF_{25} ($p<0.001$); significantly increased levelss of MDA ($p<0.001$) and LTB_4 ($p=0.003$) in Indonesian Navy divers group. There are moderate positive significant relationship between lung function changes (FEV_1) and increased levelss of MDA ($p=0.044$); and weak negative not significant relationship between lung function changes (FEV_1) and increased levelss of LTB_4 ($p=0.223$).**Conclusion**:Diving results in lung function changes, increased levelss of MDA and LTB_4 in the serum of Indonesian Navy divers. There are moderate positive significant relationship between increased lung function and increased levelss of MDA; and weak negative not significant relationship between decreased of lung function and increased levelss of LTB_4

Keywords: Indonesian Navy divers, Lung Function, MDA, LTB_4

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INTRODUCTION

Indonesia is an archipelago country comprising 17.540 islands and 2/3 of them are marine areas, the majority of population live on the littoral working as fisherman. The sea are natural resources used for the welfare of society. One of the activity carried out in the exploration of natural resources of the sea and underwater is diving. [12]

During diving, all the gas in the body has compression pressure and ambient pressure in an enclosed space is increased. The body trying to equalize the pressure between the environment and the body space, state of obstruction, the pressure can increased to the point of tissue injury. The power of this expansion and contraction result in local tissue trauma, edema and barotrauma.[9, 10,12]

On pulmonary function test of traditional divers in Semarang found that the incidence of pulmonary function impairment is 67.5%. This large number of incidence caused by lack of knowledge about the good and right technique.[20]

Various of biological substances developed as biological marker (biomarker) of oxidative stress. The substance already known and widely used as a biological marker of lipid peroxidation and oxidative stress is malondialdehyde (MDA). MDA are abundant in the circulation and constitute the main product of free radical reaction with phospholipid, produced constantly according to proportion of lipid peroxidation, so it is a good indicator to observe the speed (rate) of lipid peroxidation in vivo.[3]

Diving increase oxidative stress reaction and decompression sickness, causing damage and inflammatory reaction in the airway, as well as airway structural changes, particularly in the small airway, changes of lung function (airway obstruction) as well as the changes in the malondialdehyde (MDA) and Leukotrienes (LTB_4) concentration as an inflammation marker in the airway. The marker can be used as a sign of small airway abnormality or Small Airway Disease.[5]

Leukotrien is an inflammation mediator of eicosanoid, produced by arachidonic acid oxydation by arachidonic 5- lipoxygenase enzyme. Leukotrien divided into several types, i.e : LTA_4, LTB_4, LTC_4, LTD_4 and LTE_4. LTB_4 is a lipid mediator proinflammatory dan potensial chemoattractant recruiting leukocyte and activating the ability as well as play a role in neutrophil migration.[20]Currently in Indonesia there has been no research concerning the effects of diving toward lung function, levels of malondialdehyde (MDA) and leukotriene (LTB4) in the blood serum of diver. This study aimed to prove the influence of diving on lung function, levels of serum malondialdehyde (MDA) and leukotriene (LTB_4) on Navy divers. This study expected to provide input to the navy institution.

SUBJECT AND METHODS

This research design was a quasi-experimental to determine lung function changes, levels of serum malondialdehyde (MDA) and Leukotriene (LB_4) on navy divers result of diving that met the inclusion criteria.

The inclusion criteria consisted of a Navy diver served in the Navy diver school served in the East Surabaya. Navy diver dived in the depht of > 5 meter, sex male, 25-45 years old when the selection (first visit), has no a history of Hypertension, Asthma, COPD, willing to participate in the study after receiving an explanation and signed informed consent.

The exclusion criteria included diver had no accident in diving i.e decompression sickness and other, had no history of pulmonary operation, patient who had contraindication getting spirometry and blood samples in this study.

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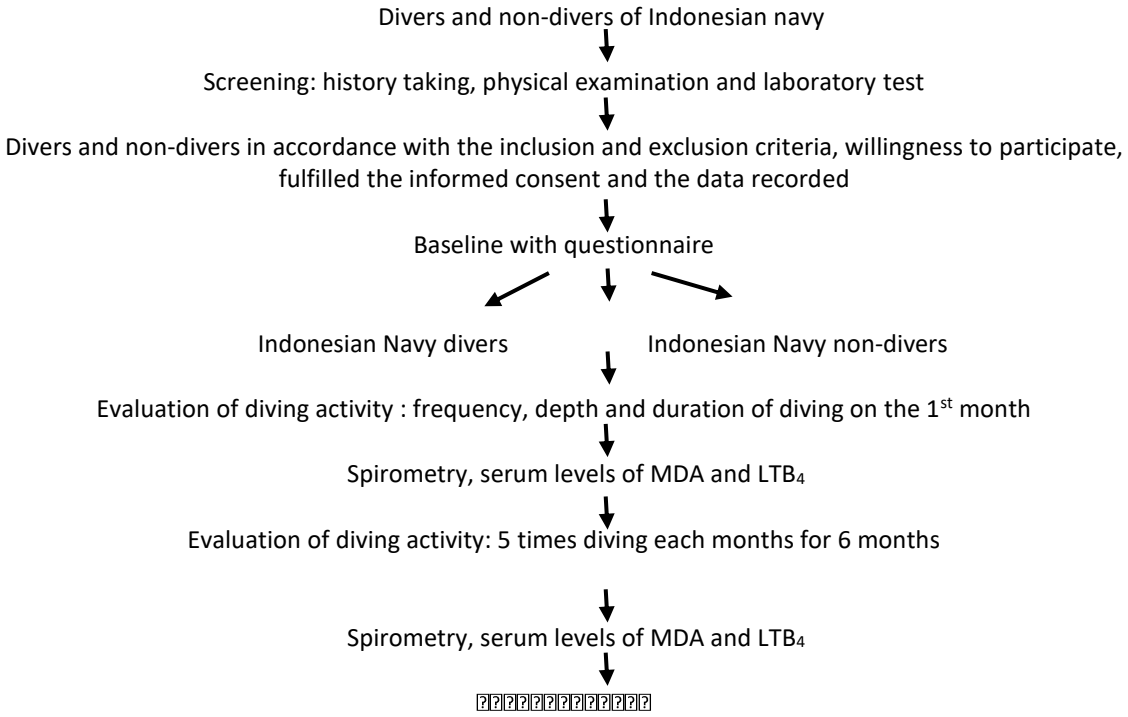

Figure 1: Flow chart

A total of 40 subjects who fulfill the inclusion and exclusion criteria recorded the basic data, carried out physical examination, laboratory and physiological pulmonary tests. The subject filled out questionnaire and conducted venous blood samples for examination of MDA and LTB_4 plasma levels using ELISA. This technique was carried out on first month Navy divers and non-divers and the end of the 6th month on Navy divers.

The data obtained was recorded on the study sheet, processed, analysed and carried out interpretation. The Statistical test used ANOVA t test and correlation test. The statistical calculation used SPSS series 17.0 (SPSS Inc., USA).

RESULTS

There were 40 people consisting of 20 Navy divers and 20 Navy non-divers and met the inclusion criteria and had willingness to participate in the study. All the patients of this study were male, non-Navy divers with the range of 30-47 years old, while the Navy divers with the mean of 25-40 years old, with the mean age of 30.75 years old. Based on level of education, the most were high school graduate about 38 people (95%), while based on the job, all members were Navy with Navy working as divers (50%) and having a certificate of diving (100%), while non-divers (50%) and didn't have a diving certificate. History of diving depth was more than 10 meters (100%). History of smoking whose still active on divers were 25% . Based on Brinkman index on active smokers on divers, the most were mild (30%), while on non-divers were 35% . The characeristics of subject presented in Table 1.

Table 1. Characteristics of Subject

Sociodemography characteristic		Non-diver		Diver		Total (n=40)	
		(n=20)	(%)	(n=20)	(%)	(n=40)	(%)
Age (years)	30 - 39	11	55	19	95	30	75
	40 - 47	9	45	1	5	10	25
Height (cm)	161 - 169	14	70	14	70	28	70
	170 - 173	6	30	6	30	12	30
Sex	Male	20	50	20	50	40	100
Levels of education	High school	18	90	20	100	38	95
	Bachelor	2	10	0	0	2	5

Job	Navy	20	100	20	100	40	100
Address	Hostel	5	25	8	40	13	32.5
	Home	15	75	12	60	27	15.3
Diving certificate	Yes	0	0	20	100	20	50
	No	20	50	0	0	20	50
Time of diving (years)	<5	0	0	3	15	3	7.5
	>5	0	0	17	85	17	42.5
History of diving depht (meter)	10	0	0	0	0	0	0
	> 10	0	0	20	100	20	50
History of smoking	Active smoker	7	35	5	25	12	30
	Former	4	20	3	15	7	17.5
	Non-smoker	9	45	12	60	21	52.5
Brinkman index	mild	7	35	6	30	13	32.5
	moderate	4	20	2	10	6	15
	heavy	0	0	0	0	0	0

The difference of spirometry using independent sample t-test concluded that there was significant difference in lung function between Navy divers and Navy non-divers. Values of FVC, FEV_1, PEF, FEF_{75} and FEV_3 in Navy divers better than non-divers, except FEV_1 / FVC.

Analysis result of independent t-test and Mann-Whitney test showed significant difference of MDA between first month non-divers and divers (p = 0.003). This may imply that there was a correlation of smoking effects on Navy non-divers that the majority were smoker with the changes of MDA levels. The levels of LTB_4 on first month divers of 0,396 (86,62 pg / mL) higher than the non-divers of 0,564 (55,72 pg/mL). While the mean levels of LTB4 between Navy divers and non-divers found no significant difference or not significant (p = 0,267). There were significant difference between first month divers and 6th month divers with P value < 0,05. But in spirometric parameter of FEV1/FVC, FEF_{2575}, FEF_{50}, FEF_{75} did not show any significant difference or not significant because it had p value > 0,05. This may imply that there was a diving effect on decreased lung function. There were difference of MDA mean levels between divers and non-divers at first month and 6th month. Result of MDA levels in first month divers was 0,576 (63.48 pg/mL), while 6th month divers was 30,365 (74,86 pg/mL). While levels of LTB_4 on first month divers was 0,396 (86.62 pg/mL) and 0,239 (162.33 pg / mL) on 6th month divers. Analysis result of paired t test showed that there was significant difference of MDA mean levels between first month divers and 6th month divers (P <0.05). Mean levels of MDA higher in 6th month divers compared with the first month divers.

Table 2: Spirometry of Indonesian navy at 1st and 6th month

Parameter	1st Diving month mean±SD	6th Diving month mean±SD	p-Value
FVC	3.91±0.35	3.58±0.40	0.001
FEV_1	3.41±0.29	3.29±0.35	0.043
FEV_1/FVC	91.77±5.32	89.16±6.48	0.08
PEF	8.77±0.87	8.05±0.76	0.001
FEF_{2575}	4.18±0.65	3.90±0.73	0.059
FEF_{25}	7.40±1.09	6.46±1.05	0.001
FEF_{50}	4.44±0.63	4.43±0.63	0.33
FEF_{75}	2.14±0.41	1.97±0.40	0.165
FEV_3	3.86±0.31	3.57±0.40	0.001

The analysis result of Wilcoxon test and paired t test showed that there was significant difference of LTB_4 mean levels between first month divers and 6th month divers (p <0.05). The mean levels of LTB_4 higher at 6th month divers compared with the first month divers.

Correlation of decreased lung function and levels of MDA using Spearman correlation test between lung function parameter with MDA levels on Navy divers at first month and 6th month obtained with the

correlation coefficient of r= 0,456 and p value = 0,044 in FEV1. It statistically showed that positive correlation was found between increased lung function and elevated MDA levels which correlated significantly in FEV1 measurenment resultCorrelation of decreased lung function and levels of LTB_4 using Spearman correlation test between lung function parameter with levels of LTB_4 on Navy divers at first month and 6th month obtained with a weak negative correlation and not statistically significant between decreased lung function and elevated LTB4 levels on divers within 6 months.

DISCUSSION

Distribution of subject according to the mean age of this study predominantly young or middle age between 25-40 years old, with a mean of 34,8 years old. Similar with the other studies [1,13,18]. All subjects of this study were male, about 40 people (100%). This is similar with the study conducted by Neubauer et al[2], 2004, in the Netherlands where all subjects were male. In contrast the subjects were male (68%) and women (32%)[1].

In this study, found decreased lung function in Navy non-divers compared with Navy divers, where the Navy divers had a number of active smokers higher (35%), while non-divers are lower (25%). Th result of spirometry among Navy non-divers and divers found a significant decreased lung function (FVC, FEV_1, PEF, FEF_{75} and FEV_3) on non-divers compared with Navy divers ($p \leq 0,05$). Nicholas et al, 2002 suggested that decreased lung function has decline in active smokers. In active smokers, there is parenchymal structure abnormality initially with chronic inflammation, resulting in parenchymal elastin destruction and elastin fibers destruction of interalveolar septum as well as increased collagen fibers caused by remodeling of lung connective tissue.

After the evaluation or observation of diving on Navy divers for 6 months at Navy divers school of Surabaya conducted every working hour start from Monday to Saturday and had to dive as much as 30 times, then showed FVC, FEV_1, PEF, FEF_{25} and FEV_3 have a lower value at the end of the 6th month compared the lung function at first month ($p < 0.05$), whereas the FEV1/FVC, FEF_{2575}, FEF_{50} and FEF_{75} also decreased, but not statistically significant ($p > 0.05$). Barnes et al., 2007 suggested that for the diagnosis of obstructive lung disease is by measuring $FEV_1/FVC < 70\%$. FEV_1 itself has meaning to the obstruction of airflow, decreased FEV_1 early even when evaluated continuously can be as an indicator of small airway disease. Other parameter used to find small airway disease is measuring decreased FVC fraction (FEF_{25-75}).

The result of statistical test using paired t-test showed a significant difference ($p < 0.05$) on decreased spirometry result of FVC, FEV_1, PEF, FEF_{25} and FEV_3 on Navy divers at the end of the 6th month. This is similar to a study conducted by Najim et al, 2006 in Iraq where in that study showed significant difference in decreased lung function due to the effect of diving. Study conducted by Dragana et al., 2009 found that the effect of diving at 42 meters in depth can decrease lung function. In study conducted by Skostad, 2007 obtained FVC value at baseline was high, during 3 years of diving obtained FVC value decreased, so does FEV_1 and FEF_{75}. Decreased lung function showed that diving result in damage to the small airway or small airway disease. In study conducted by Thorsen, 2007 suggested that changes in lung function on divers are structural changes of airway and physiological changes of lung occured after diving.

In this study the presence of elevated levels of oxidative stress demonstrated by examination of serum MDA levels at first month and 6th month in which the mean MDA at first month was 0,756 (63,48 pg/mL increased to be 378,86 pg/mL. Based on statistical analysis of serum MDA at first month was significantly increased ($p<0.001$) demonstrated that there were diving effects of increased oxidative stress. This is consistent with other studies found that diving activity result in increased oxidative stress that can be detected both locally in the airway with an examination of oxidative stress marker in the airway or using examination of exhaled breath condensate (EBC), bronchoalveolar fluid rinse and blood (Comhair et al., 2005; Dworski, 2000) . Study conducted by Tezlaff showed high levels of ROS on divers. Leimatreet et al., 2002 found an increased of NO in the diver's airway.

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Table 3: Mean levels of MDA and LTB_4 of Indonesian navy at 1st and 6th month

Group	Marker					
	MDA			LTB4		
	Mean(pg/mL)	Standart Deviation	Different test result paired t test	Mean(pg/mL)	Standart Deviation	Different test result Wilcoxon test
1st month diver	0.756	0.162	p<0.001	0.396	0.278	p=0.003
6th month diver	0.365	0.067		0.239	0.151	

This study used MDA as a marker of oxidative stress considering MDA is a lipid peroxidation product reflecting the damage induced by oxidant on unsaturated lipid in the cell membrane. MDA is one of oxidative stress marker that popular and reliable in determining oxidative stress in the clinical condition because MDA is highly reactive and toxic so underlying the relevance of using MDA as community biomedical study. MDA also more stable and membrane permeable than ROS and less toxic than other lipid peroxidation products such as 4-*hydroxynonenal* (4-HNE). Sigrid et al., 2013 found increased oxidative stress on diving, elevated plasma nitric oxide (NO), perioxtrinitrites (ONOO-) is an oxidative stress marker.

Table 4: Corelation between increased lung function and levels MDA and LTB_4 at 1st and 6th month

Spirometry	MDA		LTB_4	
	Coeficient Correlation	p value	Coeficient Correlation	p value
FVC	0.387	0.101	0.353	0.127
FEV_1	0.285	0.223	0.458	0.044
FEV_1/FVC	0.242	0.304	0.112	0.639
PEF	0.143	0.648	0.349	0.132
FEF_{2575}	0.24	0.308	0.016	0.947
FEF_{25}	0.083	0.729	0.167	0.482
FEF_{50}	0.179	0.45	0.259	0.271
FEF_{75}	0.292	0.212	0.1	0.675
FEV_3	0.328	0.168	0.397	0.087

Diving result in oxidative stress reaction, decompression sickness and gas bubbles (gas bubles) so that the damage and inflammatory processes in the airway occured. Chronic inflammation disorder in the airway involved complex interaction of cells and inflammatory mediators, most of which result in an elevated of ROS and RNS in the airway. Reactive oxygen species/ROS produced by inflammatory cells in the lung have diverse effects on airway function, including airway smooth muscle contraction, induction of airway hyperresponsiveness, hypersecretion of mucus, epithelial release and vascular exudation. ROS can also induce the production of cytokines and chemokines through the induction of oxidative stress-sensitive transcription in nuclear factor of bronchial epithelial cells.

Comparison of the results of LTB4 levels examination on 1st to 6th month divers showed that the distribution changes of elevated of LTB4 levels in the subjects. Diving can increase levels of LTB4. There is statistically significant difference between 1st month divers and 6th month divers. This is consistent with the study conducted by Jose, 2006 on smokers and nonsmokers in which the study showed no association between elevated LTB4 levels in the blood and exhaled breath condensate (EBC) between smokers and non smokers. Some factors affecting airway on diving are toxic effect result of hipoxia, microemboli caused by intravascular micro-bubbles following decompression of diving. Diving result in inflammatory reaction of small airway, pathogenesis of inflammatory proccess similar as chronic process in general.

This study showed significant positive correlation between changes in lung function and changes in levels of MDA caused by diving. Study by Cooper, 2000 suggested that physical activity enhancing immun system is physical activity that not too heavy, activity with mild and moderate intensity minimize expenditure

of free radicals, while physical activity with heavy or maximum intensity and exhausting can increase the number of leukocytes and neutrophils in the circulation and tissue.

Becher et al., 2000 concluded that chronic respiratory tract inflammation caused by diving dominated by neutrophils, there was release of proteolytic enzymes and oxidative reactions, it indicates the involvement of leukotriene in the inflammatory process of airway. LTB4 is a potent mediator of inflammatory process.

The statistically result obtained showed that there is no significant association between elevated levels of leukotriene (LTB4) and decreased lung function.

One of the characteristics of small airway obstruction is premature closure of airway, air trapping and airflow limitation. Therefore, the test conducted are useful for detecting and measuring small airway disease. *Forced Expiratory Flow* (FEF_{25-75}) part of *Forced Vital Capacity* (FVC) is a the most often spirometry variable as an indicator of small airway obstruction. As a marker of airflow limitation, FEF25-75 correlated with FEV_1/FVC, when FEF_{25-75} associated with FEV_1/FVC ratio, if FEF_{25-75} decreased, it reflects an obstruction.

Limitations of this study, among others, all of the population were male, so it can't distinguish between male and female diver. The subject on Navy divers overall were a diver who had done previous diving, found no naive Navy diver or a new diver.

SUMMARY

1. There is evidence of decreased lung function on Navy diver after observed 30 times diving for 6 months.
2. There is evidence of elevated levels of serum malondialdehyde (MDA) on Navy divers caused by diving after observed 30 times diving for 6 months.
3. There is evidence of elevated levels of serum Leukotrienes (LTB_4) on Navy divers after observed 30 times diving for 6 months.
4. There is evidence of significant positive correlation between improvement in lung function and elevated levels of MDA and not significant correlation between decreased lung function and elevated levels of LTB_4.

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