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Molecular basic of Active Smoking causes oxidative Stress (LPO) and Inflammation (Netrophil)

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Abstract :

Background : Smoking is the single largest preventable cause disease and premature death, acording to the World Health Organization. Smoking –related diseases kill one in ten adults globally, ie., 4 million deaths annualy; by 2030, if current trends continue , smoking will kill one in six people. Oxidative effects via free radical generation in smokers have been widely investigated. They cause lipid peroxidation (LPO), oxidation of proteins and damage to mainly lung and other tissues, and as is the association of smoking with increased levels of inflamatory markers. It is also well known that when smokers quit, their risk of mortality and future evevts in mainly lung and other tissues declines, but there is little data quantifying the rate of this risk reduction. Smoking triggers an immunologic response which is associted with the increased levels of inflammatory markers such as white blood cell count. Cigarette smoke can lead to a state of oxidative stress and lipid peroxidation (LPO) product manifestations in the blood and cause inflammation in the respiratory tract and skin tissues , thereby increasing the proinflammatory cytokines and causes accumulation of leukocytes netrophil.

The aim of this study: To investigated the correlation between Lipid peroxidation (LPO) and white blood cell count netrophil among active smoker.

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Methods : An observational analytic study with cross sectional approach was enroled on 36 heavy smokers during Agustus-October 2013. LPO level was measured by redox -reaction with spectrophotometry. White blood cell count (netrophyl) was determined by using an automated hematology analyzer. A Spearman correlation test was applied to analyzed the data.

Result: There is a correlation between white blood cell Neutrophyl count and LPO level (r=0.661;p=0,000)

Conclusion and suggestion : There is a positive correlation between white blood cell neutrophyl count and LPO level among aktive smoker. Need to investigated future study the effects of smoking in other stress oxidative biomarker and other damage mainly to other tissues as cardiac, lung, skin .

Key words : LPO, inflammation,stress oxidative , neutrophyl.

Introduction :

Smoking is the single largest preventable cause disease and premature death,acording to the World Health Organization. Smoking –related diseases kill one in ten adults globally, ie., 4 million deaths annualy; by 2030, if current trends continue , smoking will kill one in six people.[1]Different prevalence survey of smoking indicate that, some demographic variables sex, age, ethnicity, and socioeconomic status are consistently associated with cigarette smoking, specaially male sex, younger age, lower socioeconimic status and lower educational attainment are positively associated with smoking. The main cause of smoking starts at the age 17-20 years mainlay due to absence their neighborhood disadvantages and less likely to quiet later in life

suggesting that younger particularly vulnarable to nicotine dependence.[2] Oxidative effects via free radical generation in smokers have been widely investigated. They cause lipid peroxidation (LPO), oxidation of proteins and damage to mainly lung and other tissues, and as is the association of smoking with increased levels of inflamatory markers.[3] Cigarette smoke can lead t o a state of oxidative stress and lipid peroxidation (LPO) product manifestations in the blood and cause inflammation in the respiratory tract and damaging effects on the skin tissues, thereby increasing the proinflammatory cytokines and causes accumulation of leukocytes netrophil.[4]

Exposure to cigarette smoke increases oxidative stress buildup manifestation lipid peroxidation products (LPO) in the blood such as hydroperoxidase lipids, and superoxide anion radicals. Induction of LPO largely caused by free radical reactions involving polyunsaturated fatty acids in the membranes of biological cells. Exposure to cigarette smoke also causes inflammation that increases the proinflammatory cytokines such as Interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), and causes the accumulation of primary neutrophils leukocytes.[5]

Shifra et al and Tanni et al, say there is a relationship between oksitadive stress with an increase in neutrophils. Exposure to cigarette smoke causes an increase in macrophages thereby increasing ICAM and VCAM, as a factor in the migration of neutrophils and monocyte chemotactic to tissue, besides macrophages and endothelial cells secrete GM-GSF will stimulate the production of leukocytes granulocytes and monocytes from bone marrow. [6,7]

Is there a relationship between neutrophils and LPO in current smokers? Smokers are categorized by exposure to cigarette smoke, the number of cigarettes smoked and duration of smoking. Active smokers are those who smoke, and exposure to cigarette smoke coming from the suction smoker (mainstream). Of active smokers is classified into three parts: 1)

heavy smokers who smoked> 20 cigarettes per day in the last 5 years; 2) moderate smokers who smoked 10-20 cigarettes per day in the last 5 years; light smokers who smoked less than 10 cigarettes per day in the last 5 years. **The aim of this studyis t**o investigated the correlation between Lipid peroxidation (LPO) and white blood cell count netrophil among active smoker.

Methods : An observational analytic study with cross sectional approach was enroled on 36 heavy smokers during Agustus-October 2013. A Spearman correlation test was applied to analyzed the data. LPO examination method can be measured using a variety of methods, among others: 1) Thiobarbituric acid reactive substance (TBARS) measured by spectrophotometry at $\lambda = 532$ nm. ; 2) Malondialdehyde (MDA), which is a degradation product of LPO, and only produced by peroxidation of polyunsaturated fatty acids (PUFAs), so that the cellular lipid peroxidation such as cholesterol and acid oleic acid is not measurable. Peroxidation of polyunsaturated fatty acids decompose menghasilan malondialdehyde (MDA) and 4-hidroksialkenal. ; 3) Indirect LPO developed. The method is: LPO level was measured by redox -reaction with spectrophotometry. Where a redox reaction with the ferrous ions produce ferric ion which is then bound by a chromogen thiocyanate resulting in colors that can be measured using a spectrophotometer at a wavelength of 500 nm. [9,10,11]) Neutrophils White blood cell count (neurophil) was determined by using an automated hematology analyzer. [12] Neutrophils are also called "soldier of the body" is first deployed to the bacteria / foreign objects that enter. Neutrophils are the most of leukocytes circulating for approximately 7-10 hours, a few days after the migration into migration into the tissue. Azurofilik primary grains (lysosomes) containing acid hydrolases, myeloperoxidase, neutroamidase (lysozyme), while the secondary grains (specific) containing lactoferrin and lizozim. [13] Neutrophils have reseptpr for IgG (Fcγ-R) and complement. The first of circulating neutrophils migrate into the infected tissue quickly be

equipped with receptors like TLR2, TLR4 and another receptor. Neutrophils can recognize pathogens directly. Bonding with pathogens and phagocytosis can be increased when the antibody or complement function as opsonin binds to neutrophils. [14] Phagocytosis effective in invation germs / early foreign bodies will be able to prevent infection. In its work, phagocytic cells also complement and interact with specific immune system. [15] Antibodies as well as complement can increase phagocytosis by opsonization. Opsonin is a large molecule that is bound microbial surfaces and can be recognized by cell surface receptors of macrophages phagocyte system, thereby increasing the efficiency of phagocytosis. The final level of phagocytosis is the digestion of proteins, polysaccharides, lipids and nucleic acids in cells by lysosomal enzymes. [16].

Relationship smoke, LPO and neutrophils. Cigarette smoke produce free radicals as oxidative stress, which can damage the lipid through peroksidas process. Results peroxidation direct effect on the free radicals damage cell membranes, partly by changing the fluidity, cross-links, the structure and function of membranes, thereby causing cell death. [17] Cigarette smoke causes an inflammatory reaction, one of the inflammatory markers are leukocytes with an increase in eosinophils, neutrophils, and monocytes. Lavi et al and Canga et al found an increased number of leukocytes in heavy smokers. (18,19) Terashima et al and Walter et al mention the increased release of neutrophils and monocytes in test animals.See Figure1.[20,21]

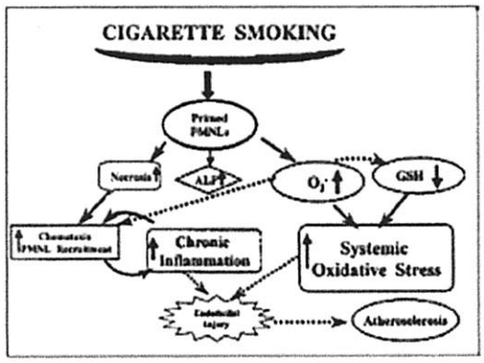

Figure 1. Relationship oxidative stress, inflammation, neutrophils. [22]

Examination conducted by taking blood neutrophils according protab venous blood sampling, m using EDTA blood specimen, and checked using automatic hematology analyzer. Data collected includes data checks LPO and neutrophils, further coding, entry and editing software by using the statistical program SPSS 11.

Result of this studies, age - average of the study subjects was 38.5 ± 9.55 years with the youngest 21 and the oldest 51 years of age. Leukocyte count in getting the results of research subjects is as much as 19.4% (an increase (> 11,000 / mm3). The results of the study subjects the absolute number of neutrophils is 1.8 to 7.8 thousand / mm3. Results LPO serum on the subject of the research showed an average 2.96 ± 1.43 µM and 0,05µM minimum and maximum values of 5.25 µm. Relations neutrophil counts and LPO r = 0.661, p = 0.000. There is a correlation between Neutrophyl white blood cell count and LPO levels. Leukocyte count in this study 6.24 thousand / mm3 - 12, 75 thousand / mm3, gained as much as 19.4% of subjects experienced an increase in the number of leukocytes (> 11,000 / mm3). The increase in the number of leukocytes in accordance with the results of research Eeden et al who reported an increase in the number of leukocytes in long-term smokers. [23] Average examination results LPO in this study with SB 2.96 ± 1.43 µM. Exposure to cigarette smoke increases the

number of neutrophils, which is in line with research Terashima et al. [24] Cigarette smoke will cause adhesion of neutrophils to endothelial cells by upregulating neutrophils and increased endothelial adhesion molecules (ICAM). The increase was also caused by a neutrophil chemotactic factor (IL-8, Leukotrienes B4) which is released by endothelial cells in response to inflammation. Expression of IL-8 by endothelial cells occurs due to activation of MAP kinase and transcription factor NF-κB which will serve as the primary regulator of cytokine production and expression of factor chemotaktin. This is consistent with Morrison et al who reported there was an increase of neutrophils in smokers after 12 hours of exposure to smoke. [25]

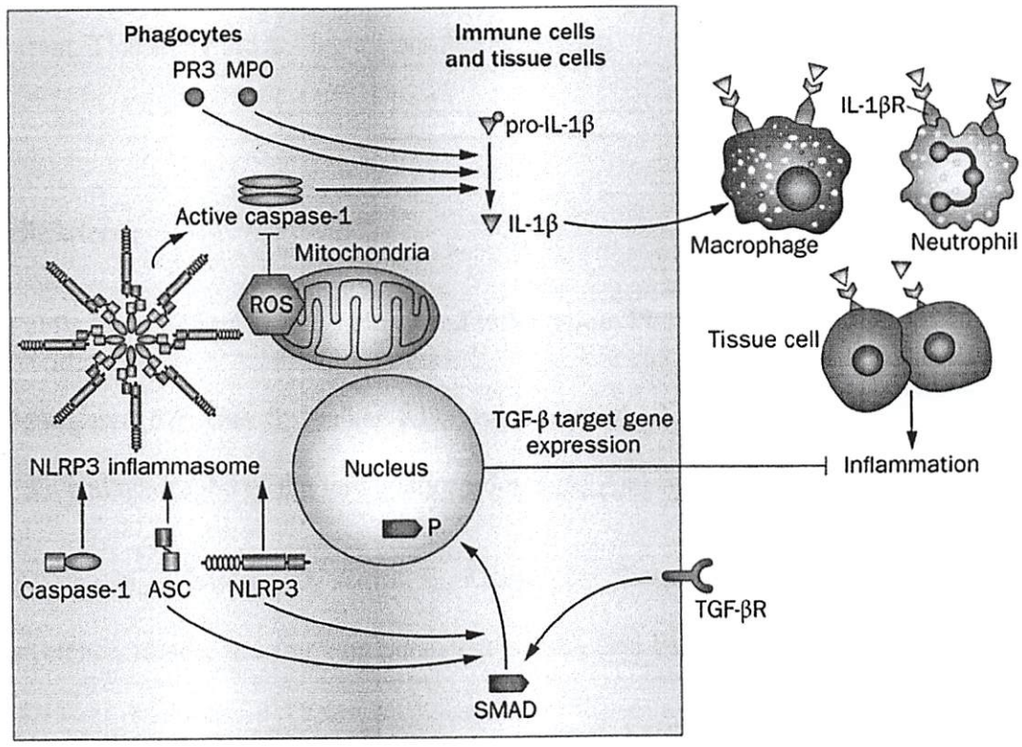

Figure 2. Immunoregulatory role of ROS, and neutrophil. Modificated 26)

Exposure to cigarette smoke causes oxidative stress and cause inflamasi.Study proves the existence of a correlation between oxidative status through LPO parameters with neutrophils as

one of the signs of inflammation. NLRP3 and ASC form the NLPR# inflammation, which activates caspase-1. Cleavage of Pro-IL-1β by caspase 1 or neutrophil serine proteases (PR3 or MPO) generates mature IL-1β, which is releaseed into the extracellular space. Binding of IL-1βto IL-1βR expressed on immune cells or parenchymal tissue cells leads to inflammation. See Figure 2.[26] There is a positive correlation between the white blood cell count and LPO level neutrophyl Among aktive smokerNeed to future study investigated the effects of smoking in biomarkers of oxidative stress and other damage mainly to other tissues as cardiac, lung, skin.

This study already has obtained ethical clearance and approval by the patient through informed consent. This study did not have a conflict of interest.

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