

Malondialdehyde Meningkatkan Tingkat Antigen Khusus Prostat di Antara Pekerja Bengkel Mobil

MALONDIALDEHYDE INCREASES LEVELS OF PROSTATE SPESIFIC ANTIGEN AMONG AUTO BODY REPAIRED WORKERS

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Abstract

Cadmium (Cd) is one of the environmental factors that can cause prostate cancer. Cadmium can induces metallothionein (MT) in several tissues that functions to carry out an intracellular detoxification procession by binding to Cd. Exposure of Cd can cause an increase in reactive oxygen species which will cause lipid peroxidation with the end product being malondialdehyde (MDA). The research aims to determine the correlation between MT, MDA and prostate specific antigen (PSA) among auto body repair workers. The study used a cross sectional design involving 32 auto body repair workers and their ages ranged from 18 to 66 years. Measurement of MT, MDA and PSA using the ELISA method. We performed a multiple regression analysis to evaluate the relationships between MT, MDA with PSA for age and other covariates. The mean age of subjects was 38.88 ± 12.82 years. The mean levels of MT was 2.84 ± 1.18 ng/dL, MDA was 8.22 ± 20.06 ng / dL and PSA was 0.86 ± 1.79 ng / dL. The study found that MT ($\beta = 0,232$; 95% C.I. 0,202 – 0,503; $p < 0,001$) and MDA ($\beta = 0.827$; 95% CI. 0.065 – 0,083; $p < 0,001$) significantly predicted PSA. MDA ($\beta = 0.827$) has stronger relationship with PSA compared to MT ($\beta = 0.232$). This results confirmed the role of MT and MDA on PSA synthesis. Metallothionein induce the process of lipid peroxidation with the end product being malondialdehyde (MDA) and increase PSA.

Keywords : auto body repair workers, metallothionein, malondialdehyde, prostate

Abstrak

Kadmium (Cd) merupakan salah satu faktor lingkungan yang dapat menyebabkan kanker prostat. Kadmium dapat menginduksi metalotionin (MT) di beberapa jaringan yang akan berikatan dengan Cd untuk proses detoksifikasi. Paparan Cd dapat meningkatkan ROS yang akan menyebabkan terjadinya peroksidase lipid dengan hasil akhir malondialdehid (MDA). Penelitian bertujuan untuk mengetahui hubungan antara MT, MDA dan PSA pada pekerja bengkel mobil. Penelitian menggunakan desain kros sektional dengan subyek sebanyak 32 pekerja bengkel mobil yang berusia 18 hingga 66 tahun. Pengukuran MT, MDA dan PSA menggunakan metode ELISA. Analisis statistik menggunakan analisis regresi berganda. Rata-rata usia subyek adalah 38.88 ± 12.82 tahun. Rata-rata kadar MT 2.84 ± 1.18 ng/dL, MDA 8.22 ± 20.06 ng / dL dan PSA 0.86 ± 1.79 ng / dL. Hasil uji didapatkan MT ($\beta = 0,232$; 95% C.I. 0,202 – 0,503; $p < 0,001$) dan MDA ($\beta = 0.827$; 95% CI. 0.065 – 0,083; $p < 0,001$) berpengaruh terhadap PSA secara signifikan. MDA ($\beta = 0.827$) mempunyai hubungan dengan PSA yang lebih kuat dibanding MT ($\beta = 0.232$). Hasil penelitian mengkonfirmasi adanya peran MT dan MDA pada sintesis PSA. MT menginduksi proses peroksidase lipid dengan produk akhir MDA dan meningkatkan kadar PSA.

Kata kunci : pekerja bengkel mobil, metalotionin, malondialdehid, prostat

INTRODUCTION

Prostate cancer is the most common visceral cancer in men. This cancer can be caused by environmental factors. One of the environmental factors which is a risk factor for prostate cancer is heavy metal exposure including cadmium (Cd). Our previous studies have found positive differences between blood cadmium levels and prostate specific antigen (PSA) levels. The results also found a significant percentage increase in PSA in welding workers compared to office employees.¹ Cadmium can induce metallothionein (MT) in several tissues, including the liver and kidneys. Metallothionein, as a low molecular weight intracellular protein, functions to carry out an intracellular detoxification process by binding to Cd. Metallothionein also plays a role in the gastrointestinal absorption of Cd, Cd retention in tissues and decreases biliary excretion of Cd. In acute toxicity, liver is the primary target, whereas in chronic toxicity, kidneys are major targets of Cd. In chronic intoxication, Cd stimulates de novo synthesis of MT; it is assumed that toxicity in the cells starts when loading with Cd ions exceeds the buffering capacity of intracellular MT. Study showed a significant correlation between urine Cd and MT.^{2,3} Study by Nakazato et al investigated the normal range of MT1/2 in 200 healthy human serum and found it to be 27-48ng/ml.⁴

Many studies have shown that MT expression varies in different tumors, suggesting that MTs may play a vital role in carcinogenesis^{5,6}. In a study to compare PSA and MT levels in blood serum of 46 prostate cancer-diagnosed screening by biopsy demonstrated that PSA levels vary significantly and in contrary, MT levels were elevated.⁷ In Indonesian, PSA increased with ageing on men with benign Prostatic Hyperplasia. These results are consistent with results in other sets of population and vary between different countries and ethnicities. The indications for prostate biopsy were PSA > 4.0 ng/mL.⁸

Exposure to cadmium can cause the production of reactive radicals such as superoxide anion radicals and nitric oxide in biological systems.⁹ The amount of superoxide anion which increases due to Cd exposure shows a promotional effect on cancer cells by facilitating

cellular immortality through cell resistance to apoptosis and signal transduction in cancer cell proliferation, such as prostate cancer.^{10,11} This will cause decrease glutathione, change antioxidant enzymes activity and change in the structure of membrane cells through the process of lipid peroxidation with the end product being malondialdehyde (MDA).¹² Malondialdehyde (MDA) is an end product of the oxidation of polyunsaturated fatty acids and it has been used as a marker for oxidative stress.¹³ Serum level of MDA may be used as a marker in screening for prostate cancer as a complement to PSA, serum MDA($\mu\text{mol/l}$) was observed to be significantly higher in CaP(2.05 ± 0.64) when compared with control (0.06 ± 0.09).¹⁴

Study in the freshwater crab *Sinopotamon henanense* demonstrated MT contents increased after single Cd exposure and also showed a time- and dose-dependence. so that the MDA contents increased with exposure time. The results demonstrated that the MDA contents increased with exposure time and dose and showed time- and dose-dependence in both gills and hepatopancreas of *S.henanense* after single Cd exposure.¹⁵

Auto body repaired workers are often exposed to metal fume from workplace environment. The formation of fume is due to the evaporated metal coming in contact with oxygen and producing metal oxides which then condense and form fume. Metal oxide particles that can be present in metal fumes are Aluminum (Al), cadmium (Cd), cobalt, chromium (Cr), copper (Cu), fluorides, iron (Fe), lead, manganese (Mn), magnesium, molybdenum, nickel (Ni), silica, titanium, and zinc (Zn). International Agency for Research on Cancer (IARC) has recently classified welding fumes as group I carcinogens.¹⁶ The action level for workplace exposure to cadmium is 5 micrograms per cubic meter of air ($5 \mu\text{g}/\text{m}^3$) calculated as an 8-hour time-weighted average (TWA) exposure. The PEL is a time-weighted average concentration that must not be exceeded during any 8-hour work shift of a 40-hour work week.¹⁷ Cadmium is absorbed by the intestinal mucosa and bound to metallothionein. Cadmium-metallothionein complex is transported to the target organs. Metallothionein levels are increased in several

tumors including breast, colon, kidney, prostate, ovary, nasopharynx, bladder, salivary glands, testicular and thyroid tumors. This unique protein is involved in a variety of intracellular functions, but its role in detoxification of heavy metals and in maintaining homeostasis of essential metal ions, which are caused by high affinity for these metals, has been largely investigated.^{18,19} A study involving 295 men, 50 years of age and above showed the PSA levels are reversely associated with blood Cd and urine Cd levels. A direct role for cadmium in our study as an inductor of PSA expression cannot be excluded.²⁰ Whether increased PSA in auto body repaired workers affected by MT is still unclear, and the role of MT and MDA in PSA synthesis is still unknown. This study aims to determine the correlation between MT, and MDA with PSA among cars workshop workers.

METHODS

Research design

The study used a cross sectional design, located at Banyumas in October 2019.

Research subjects

Research subjects were 32 cars workshop workers taken by purposive sampling with inclusion criteria who had worked for at least 6 months and agreed to sign informed consent. The number of subjects was calculated using $\alpha = 0.01$, $\beta = 0.05$ and $r = 0.7^{14}$.

Determination of MT, MDA and PSA levels

MT, MDA and PSA was measured using blood plasma taken from vena cubiti. The blood samples were obtained between 09:00–11:00 a.m. from each subjects following 12-h fasting. The venous blood samples were collected into heparinized tubes and centrifuged at 3000 revolution per minute for 10 minute. The separated serum samples were stored at -80°C until the day of laboratory investigation of MDA, MT and PSA. Determination of MT, MDA and PSA using the ELISA assay based on the principle of sandwich ELISA. The microtiter wells are coated with an antibody, directed towards an epitope of an antigen molecule. An aliquot of serum is incubated in the coated well

with enzyme conjugated second antibody (E-Ab), directed towards a different region of the antigen molecule. After incubation the unbound E-Ab is washed off. The amount of bound E-Ab is proportional to the concentration of antigen in the sample. After adding the substrate solution, the intensity of colour developed is proportional to the antigen concentration in the sample. The measured Optical Density of the standards are used to construct a calibration curve against which the unknown samples are calculated The absorbance value (OD) was measured at 450 nm.

Statistical analysis

Data analysis using multiple regression analysis, with PSA was the dependent variable, age, MDA and MT were the independent variables. A value of $p < 0.05$ was accepted as statistically significant.

Ethical clearance

Ethical approval was granted by the Medical Research Ethics Commission Faculty of Medicine Jenderal Soedirman University Ref : 6235/KEPK/IX/2019

RESULTS

Study held on 32 subjects cars workshops characteristics as shown at table 1. According to the Table, the average of age was $38,88 \pm 12,82$ years, MT was $2,84 \pm 1,18$ ng / dL, MDA was $8,22 \pm 20,06$ ng / dL and PSA was $0,86 \pm 1,79$ ng / dL.

Table 1. Distribution of Age and Clinical Features of the Subject (n=32)

Characteristic	Mean±SD
Age (yrs)	38.88 ± 12.82
MT (ng/dL)	2.84±1.18
MDA (ng/dL)	8.22±20.06
PSA (ng/dL)	0.86±1.79

Table 2. Spearman Rho Test

Variable	PSA (ng/dL)
Age (yrs)	$r = -0.124$
$p = 0.500$	
MT (ng/dL)	$r = 0.426$
$p = 0.015$	
MDA (ng/dL)	$r = 0.627$
$p = 0.000$	

Multiple regression analysis was applied to predict PSA from MT, MDA and age. MT ($\beta = 0,232$; 95% C.I. 0,202 – 0,503; $p < 0,001$) and MDA ($\beta = 0,827$; 95% C.I. 0,065 – 0,083; $p < 0,001$) significantly predicted PSA. MDA ($\beta = 0.827$) has stronger relationship with PSA compared to MT ($\beta = 0.232$).

Table 3. Multiple Regression Analysis With PSA as Dependent Variable

	Unstandardized Coefficients		Standardized Coefficients	95% Confidence Interva	
	B	Std. Error	Beta	Sig.	Lower Bound
Constant	-0.877	0.322		0.011	
MT	0.352	0.074	0.232	<0.001	0.202
MDA	0.074	0.004	0.827	<0.001	0.065
Age	0.003	0.006	0.023	0.578	-0.008

DISCUSSIONS

The results of MT research on prostate cancer are conflicting. One study showed that MT was significantly lower in tumor tissue, another identified MT levels that increased significantly. In addition, a study based on radioimmunoanalysis revealed insignificant decreased MT levels in tumor tissue. Therefore more research is needed.²¹

The results of the current study revealed positive relationship between MT and PSA. Study by Gumulec et al. showed significantly enhanced MT level in prostate cancer patients.²² These findings indicate possible alternative role of MT to PSA prostate cancer marker. Antiapoptotic, antioxidant, proliferative, and angiogenic effects of metallothionein (MT) -I + II show the role of MT in oncogenesis, tumor development, therapeutic response, and patient prognosis. Research has reported an increase in MT-I + II in various human cancers; such as breast, kidney, lung, nasopharynx, ovary, prostate, salivary glands, testes, bladder, cervix, endometrium, skin carcinoma, melanoma, acute lymphoblastic leukemia (ALL), and pancreatic cancer. However, MT-I + II decreases in other types of tumors (eg, hepatocellular, gastric, colorectal, central nervous system (CNS), and thyroid cancer).²³ In a study examining the systematic comparison of PSA and MT levels

in the blood serum of patients diagnosed with prostate cancer, MT levels were increased in all samples tested including patients with PSA levels of 0-4 ng / dL and no significant variations were observed. These results indicate the potential for MT as a marker of additional prostate cancer which reduces, in combination with PSA, the possibility of false positive / negative diagnosis.⁷

Oxidative stress is known as one of the mechanisms that trigger prostate cancer development and the development of prostate hyperplasia. Oxidative stress has been linked to the development of benign prostate hyperplasia and the development of prostate cancer. Reactive oxygen species (ROS) can indirectly cause random DNA formation by triggering autocatalytic lipid peroxidation, which produces various genotoxic potential substances for breakdown products, including alkoxy radicals, peroxy radicals, and aldehydes, such as malondialdehyde.²⁴

Long-term exposure and accumulation of heavy metals in the body can disrupt oxidative stress genes and thus increase susceptibility to various diseases. The results of research on fishermen in heavy metal polluted areas showed high concentrations of MT in accompanied by high metal concentrations such as cadmium (Cd), copper (Cu), lead (Pb), chromium (Cr), and zinc (Zn). At the same time, a significant decrease in glutathione content and catalase enzyme activity was associated with a significant increase in the concentration of malondialdehyde in fishermen's serum.²⁴

Research on smelting workers found increased levels of MDA and MT compared with the control group. Concentration of MDA in the plasma of smelters was higher in comparison with its concentration in the non-smoking control group. The plasma and the erythrocyte lysate MT concentration increased significantly in the whole group of smelters as compared to the non-smoking control group. The mean value of MT concentration in plasma of the smoking smelters was above 2-fold higher than in the non-smoking control group.²⁵

Study by Dilioglulil et al. found significant relationship between PSA and blood and tissue MDA levels.²⁶ This report is in line with our study, that MDA and PSA has positif correlation. Status of oxidative stress in the prostate is

characterized by increased levels of MDA, decreased glutathione (GSH) concentrations and decreased cytosolic enzyme activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione S-transferase.²⁷

CONCLUSION

This study demonstrated that MT and MDA has a role in PSA synthesis. Metallothionein induce the process of lipid peroxidation with the end product being MDA and increase PSA.

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