

was felt necessary to conduct another study on the association of smoking with MDA and GSH concentrations in smokers.

METHODS

Research design

This was an analytical observational study with cross-sectional approach that was conducted from April until December 2015 in the medical study program of the Faculty of Medicine, Sriwijaya University, Palembang, South Sumatra.

Study subjects

The study sample comprised all smoking students aged 18-22 years in the medical study program of the Faculty of Medicine, Sriwijaya University, who met the inclusion criterion and did not meet the exclusion criteria. The inclusion criterion was healthy males, while the exclusion criteria were students who were ill or had a history of a metabolic disorder, such as diabetes mellitus and cardiac and renal disorders. The subjects to be included in the sample were recruited by identifying all smokers and selecting the subjects according to the inclusion criterion. The sample size was determined using Slovin's formula,⁽¹⁵⁾ namely $n = N / (1 + N \times e^2)$, where N is the population size, and e is the margin of error. Applying a 5% error margin in Slovin's formula, the recommended minimum sample size was 40. The sample size that was obtained in this study was 20 smoking students and 20 nonsmoking students. The study subjects were selected using simple random sampling by lot after those involved agreed to participate in the study and had been given an explanation of the benefits and discomforts of the participation in this study. Their willingness to participate in the study was recorded by signing informed consent.

Data collection

Collection of the sample data was performed by interviews using a questionnaire,

which included the subjects' identity (using initials only), age, gender, duration of smoking, and number of cigarettes per day.

Laboratory analysis

A blood sample of 2 ml was drawn at 07.30 AM West Indonesian Time from the left median cubital vein of each (nonfasting) study subject using a 3-mL syringe. The blood samples were then centrifuged at 5000 rpm for 2 minutes to obtain plasma, which was then stored at -20°C, before being used for measurement of GSH and MDA concentrations.

The plasma GSH and MDA concentrations were determined in the Biochemistry Laboratory and the Molecular Biology Laboratory, Faculty of Medicine, Sriwijaya University. The plasma GSH and MDA concentrations were measured biochemically using the Sigma GSH and MDA assay kits, respectively.

Determination of plasma GSH concentrations was done biochemically using the Sigma GSH assay kit. A volume of 200 μ l plasma was deproteinized with 200 μ l of 5% 5-sulfosalicylic acid (SSA). The mixture was then vortexed and left standing for 10 minutes at 2-8°C. Subsequently the mixture was centrifuged at 10,000 g for 10 minutes to remove the precipitated protein. Then 10 μ l of the supernatant was taken and mixed with 150 μ l working mixture in each well of a 96-well plate. The mixture was incubated for 5 minutes at room temperature, then 50 μ l of NADPH solution was added and the absorbance was measured using a spectrophotometer at a wavelength of 412 nm. The GSH concentration was then determined on the GSH standard curve in units of μ mol/L.

Determination of plasma MDA concentrations was done biochemically using the Sigma MDA assay kit. A volume of 10 μ l plasma was mixed with 500 μ l of 42 mM H_2SO_4 and 125 μ l phosphotungstic acid in a microcentrifuge tube. The mixture was vortexed and incubated at room temperature for 5 minutes, then centrifuged at 3,000 g for 3 minutes. The precipitate was