CHAPTER III

METHODS

Subjects

The study tentatively included 17 normal subjects, 17 COPD patients with anemia, and 17 COPD patients without anemia from the internal medicine outpatient clinic, Maharajnakorn Chiang Mai Hospital, Chiang Mai University. Both male and female COPD patients were recruited and all were clinically stable, without any change in medication during the previous three weeks. They were classified as stage II-IV according to GOLD COPD guidelines ($FEV_1/FVC < 0.70$; $30\% < FEV_1 < 80\%$) (1). Anemia was defined as hemoglobin concentration below 12 g/dl in women and 13 g/dl in men. All subjects in each group were matched in age, weight, height, and gender.

The patients were excluded from this study if they 1) required oxygen therapy, 2) used medication affecting exercise performance such as β-blocker, 3) had conditions that limited exercise: heart diseases, hypertension, diabetes mellitus, musculoskeletal disorder, and neurological diseases, 4) did not understand verbal communication, 5) had impaired vision and hearing after correction, or 6) had known vitamin B12 or folic acid deficiency.

Procedures

Prior to the study, written informed consents were obtained. Blood sample tests, the spirometric maneuvers, and the 6MWT were performed on all subjects. All the tests were completed in one session. This study was begun in the morning and the subjects were asked to not to have alcohol, caffeine, or perform any vigorous exercise within 2 hours before the test.

The subjects reported to the laboratory in the morning, and demographic data such as age, weight, and height were measured. After resting for 5 minutes, blood pressure and heart rate were recorded and the testing procedures were explained to the subjects.

5 milliliter peripheral venous blood samples were collected by nurse. A standard hemoglobin test was performed, According to WHO guidelines (30). This result was used to identify individuals' anemic condition.

Afterward, FVC, FEV_1, and FEV_1/FVC were measured by using a spirometer (Spirolab II, Italy). All subjects were asked to sit in upright position with head slightly elevated. Their noses were occluded by nose clips. They were asked to tightly seal their mouth around the mouth piece connected to the spirometer. They were instructed to breathe normally for at least three times and then forcefully and deeply inhale as much as they could. Once the lungs were completely filled, they were asked to forcefully and rapidly exhale for at least six seconds. According to the ATS spirometry guidelines, the test maneuver was performed for at least three times but no more than eight times, with adequate rest in between (49). The best value of each variable was used for further data analysis.

Next, the standardized 6MWT (10) was performed by a physical therapist who did not know to the hemoglobin level and pulmonary function test results. The 6MWT was performed over a 20-m corridor on the 2nd floor of Sujinno building, Maharajnakorn Chiang Mai hospital, Faculty of medicine, Chiang Mai University, Chiang Mai. Subjects were asked to walk from the starting point to the end point. They were asked to cover as much as distance as possible in six minutes. The subjects walked as fast as possible but running was prohibited. All subjects were permitted to slow down, to stop, or to rest as needed during the test. The same verbal encouragement "do your best" at the start of each minute was provided to all subjects. If the subjects took a rest, the verbal encouragement "begin walking as soon as you feel able" was given. Before and immediately after the test, the heart rate, the blood pressure, the oxygen saturation, the dyspnea scale, and the leg fatigue perception were recorded. The heart rate and oxygen saturation were recorded at rest and immediately after the test using a pulse oxymeter (MASIMO™, CA, USA). The blood pressure was measured by using sphygmomanometer (HM-1100, Japan). The modified Borg scale of 0 to 10 was used to rate dyspnea and leg fatigue perception at rest and after the test.

In addition, the total walking distance covered in 6 minutes was recorded after the test. The second trial of 6MWT was repeated after 30 minutes rest, or after the heart rate returned to baseline. The best results of the 6MWT were used for further analysis. The test was terminated if the subjects experienced chest pain, intolerance dyspnea, leg cramps, staggering, diaphoresis, and pale or ashen appearance.

Statistical analysis

Descriptive statistics were used for reporting demographic data, spirometry results, heart rate, oxygen saturation, and 6MWD. A one-way repeated measure ANOVA was used to compare the differences among the normal subjects, COPD patients with anemia and without anemia. If any significant differences were found, a post hoc analysis using Scheffe''s test was performed. All statistical analysis was done using SPSS software program (Version 11.5). Data were reported as mean ± SD.