

## The effect of excessive disodium ethylene diamine tetraacetic acid (Na<sub>2</sub>EDTA) anticoagulant concentration on leukocytes profile in peripheral blood examination

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### Abstrak

Akurasi pemeriksaan profil lekosit dipengaruhi beberapa faktor preanalitik diantaranya konsentrasi antiokoagulan. Antikoagulan yang paling sering dipakai pada pemeriksaan darah rutin adalah EDTA. Ketidaksesuaian perbandingan konsentrasi EDTA dengan bahan darah berefek terhadap hasil pemeriksaan darah tepi diantaranya parameter lekosit. Penelitian ini bertujuan untuk mengetahui apakah terdapat perbedaan antara hasil pemeriksaan profil lekosit pada bahan darah dengan berbagai konsentrasi antikoagulan Na<sub>2</sub>EDTA yang berbeda. Penelitian ini merupakan penelitian potong lintang. Bahan penelitian berupa 33 sampel darah vena mahasiswa Fakultas Kedokteran UGM Yogyakarta. Dua mL darah dibagi ke dalam 4 tabung Na<sub>2</sub>EDTA. Tabung pertama berisi Na<sub>2</sub>EDTA konsentrasi standar, 2 mg/dl, tabung yang lain secara berurutan berisi Na<sub>2</sub>EDTA dengan konsentrasi 4 mg/dl, 6 mg/dl, and 8 mg/dl. Sebelumnya dibuat sediaan hapus langsung dari setetes darah tanpa antikoagulan (sebagai kontrol). Darah dalam keempat tabung tersebut segera dilakukan pembuatan sediaan hapus dan diperiksa profil hematologi lekositnya menggunakan SYSMEX SE-9500 automatic analyzer. Terdapat perbedaan yang bermakna dari hitung lekosit, hitung jenis lekosit absolut dan prosentase monosit pada konsentrasi Na<sub>2</sub>EDTA yang berlebihan. Prosentase netrofil relatif meningkat dan terdapat perbedaan yang bermakna. Prosentase limfosit, eosinofil dan basofil tidak berbeda secara bermakna. Pemeriksaan morfologi lekosit menunjukkan perubahan yang bermakna berupa tepi sitoplasma yang irreguler, vakuolisasi, dan lobus nukleus yang irreguler akibat pengaruh konsentrasi Na<sub>2</sub>EDTA yang berlebihan. Disimpulkan bahwa penggunaan konsentrasi Na<sub>2</sub>EDTA yang berlebihan pada preparasi spesimen darah menyebabkan perubahan profil lekosit sesuai peningkatan konsentrasinya. Konsentrasi standar tidak mempengaruhi hitung lekosit dan hitung jenis lekosit serta morfologinya, kecuali berpengaruh terhadap tepi sitoplasma yang irreguler dan lobus nukleus yang irreguler. (Med J Indones 2007; 16:168-75)

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Accuracy of leukocytes profile assessment is influenced by several pre analytical factors, among others, the anticoagulant concentration. EDTA is one of the most frequently used anticoagulant in peripheral blood examination. Several references stated that inappropriate concentration of EDTA anticoagulant in blood sample may affect the result of leukocytes profile in peripheral blood examination. The aim of this study was to evaluate whether there are differences among leukocytes profile in peripheral blood examination specimens, which were prepared with excessive Na<sub>2</sub>EDTA anticoagulant in different concentration. This study was conducted in Faculty of Medicine, Gadjah Mada University. Blood samples from 30 subjects were taken using vein puncture. Two millimeters blood was divided into 4 Na<sub>2</sub>EDTA-containing tubes. Before that, one drop of blood without Na<sub>2</sub>EDTA anticoagulant was used to make blood film right after vein puncture, as control. Each tubes contained different concentration of anticoagulant. The first tube contained Na<sub>2</sub>EDTA in standard concentration 2 mg/ml; the remaining tubes contained 4 mg/ml, 6 mg/ml, and 8 mg/ml respectively. These samples were immediately examined using SYSMEX SE-9500 automatic

cell counter to measure the total and differential leukocytes count; and were stained with Wright staining for morphological examination under the microscope. These procedures were done before 20 minutes of vein puncture. There were significant decrement of total leukocytes count, absolute differential leukocytes count and monocyte percentage following excessive Na<sub>2</sub>EDTA administration. Neutrophil percentage was found to be relatively increased and the difference was significant. Lymphocyte, eosinophil and basophil percentages were not significantly different. Morphological examination showed significant increment in irregular cytoplasm margin, vacuolation and irregular nuclei lobes following excessive Na<sub>2</sub>EDTA administration. It is concluded that excessive concentration of Na<sub>2</sub>EDTA used in blood specimen preparation, will lead to changes in leukocytes profile as the concentration increased. Standard Na<sub>2</sub>EDTA anticoagulant concentration did not alter any leukocytes count and morphology, except for irregular cytoplasm margin and irregular nuclei lobes. (Med J Indones 2007; 16:168-75)