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The influence of protein fractions and electrolyte imbalance on refractive index of serum in patients with multiple myeloma

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Abstract. Refractometric analysis is very rapid, accurate and simple method of analysis measuring refractive index of biological liquids such as serum, plasma, spinal fluid, urine. This method can be used for definition total protein and solids concentrations in serum. The value of refractive index depends on all substances in serum including proteins, lipids as well as low molecular weight compounds, for example ions of different metals.

Refractometric analysis shows strong correlations between protein concentrations in serum of patients with multiple myeloma and its(serum) refractive index which depends on protein concentration and doesn’t depend on electrolyte disbalance.

1. Introduction

Multiple myeloma (MM) is a lymphoproliferative neoplasm with clonal plasma cells substrate. Multiple myeloma refers to a group of paraproteinemic hemoblastosis with typical produce and secrete a monoclonal immunoglobulin and/or its free light chains in serum and/or urine, seldom in cytoplasm of clonal plasma cells only [1, 2, 3]. Multiple myeloma accounts for about 1% of all types of malignancy and slightly more than 10% of hematologic malignancies [4]. The disease is considered incurable despite of advances in treatment and diagnostics [4, 6, 7].

There are different immunochemical types of MM which defined by types of involved secreting immunoglobulin (Ig): IgA-MM, IgG-MM, IgD-MM, IgE-MM, IgM-MM, Bence-Jones myeloma (BJ-MM), diclonal myeloma, nonsecretory myeloma and light chain myeloma [1, 2, 8, 11].

The increasing of calcium (total and ionized) and phosphorus concentrations are markers of active bone demineralization and bone destruction in MM patients with high monoclonal protein, especially for III stage of disease [8, 11].

Our research demonstrates differences in refractive index between control (donors) and experimental (patients with MM) groups with typical protein and electrolyte disturbance.
2. Materials and methods

2.1 Blood samples.
S-Monovette tubes were used (Sarstedt, Germany) with clotting activator for receiving of serum samples. The blood samples collected to tube and put in a support in room with +18-24°C within 20-30 min. After that serum was received by centrifugation within 15 min at 3,000 rpm (Thermo Scientific Heraeus Labofuge 200, USA). The samples were frozen (−30°C) and kept for refractometric analysis.

2.2 Refractometric analysis.
Serum were researched on refractometer (Anton Paar Abbemat 200, Austria) for dimension of total solids and protein concentrations in control group and patients with MM. Refractive index was measured on wavelength λ=589 nm, temperature T=17.5°C by experimental method. The accuracy of refractive index measurement is ±0.0001.

The sample was applying on the surface of prism and lit by LED illumination through an interference light filter with use different light angles and directions. The reflected light beam was recorded by matrix sensors. Information was processed by refractometer and displayed in automatic mode.

3. Results and discussions
Serum samples was researched from 14 patients with MM (stage II and III, age 42-79 years old), who was observed in Hematology Clinic of Russian Scientific Research Institute of Hematology and Transfusiology. Fifty-five serum samples from healthy donors entered into control group.

Refractometric analysis of total protein concentration in serum based on extent of change of light beam. The value of refractive index of serum depends on quantity and physic-chemical characteristics of being proteins in serum. It is bound to low depend of electrolytes and non-protein organic substances on refractive power because it has constant concentrations in healthy donors serum. Imbalance in calcium-phosphorus system is typical for MM patients and it leads to increase of concentrations these elements in serum and urine [8, 11]. Patients with MM and control group can have strong difference in concentration of electrolytes (calcium and phosphorus) owing to bone destructions in MM patients. Calcium ions can bound to monoclonal protein, that decrease differences between protein and solids concentrations in serum for some patients. [15]. This feature is shown on Figure 1-b (refractive index 1.3544 D and 1.3556 D). Never the less there is still the reliable differences remain between two groups (donors and MM patients) in concentrations of electrolytes and total protein.

M-protein is not secreted in some patients, who have specific forms of MM such as “nonsecretory” myeloma, Bence-Jones myeloma and light chain myeloma [1, 2, 11]. Aforementioned types of MM have not M-protein in serum. Our experimental data confirm this feature (see the points with refractive index 1.3462 D, 1.3483 D and 1.3521 D in Figure 1 (a, b)). M-protein is absent in serum of MM patients with nonspecific types (Figure 2).
Figure 1. Dependence serum protein (a) and total solids (b) concentration on the refractive index. Red squares show MM patient’s data and green triangles demonstrate donor’s data.
4. Conclusion.
The data of refractometric analysis show differences between patients with MM and control group (donors) in protein and electrolytes (calcium and phosphorus) concentrations in serum and their refractive index. Patients with MM have higher scores on all aforementioned features. The value of refractive index primarily depends on total protein and M-protein concentrations for patients with multiple myeloma as well as for healthy donors. Refractometric analysis can be use as one of suitable method of detection changes in protein concentration in MM patient’s serum.

References