The Electrophoretic Patterns of Serum Proteins in Children

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Protein electrophoresis is a specific, simple and reliable method used for separation of serum proteins. The purpose of this study was to detect at the subjects group, pathological patterns of serum proteins by electrophoresis on cellulose acetate, as well as to interpret any abnormalities The study was performed on 966 sera collected from pediatric subjects admitted to Emergency Hospital for Children Sfantul Ioan in Galati for various symptomatology. Study results revealed 333 specific pathological electrophoretic pattern for subacute, acute and chronic inflammatory response (164 children), hypogammaglobulinemia (67), polyclonal hypergammaglobulinemia (24), nephrotic syndrome (3), protein losing enteropathy (7), hypoalbuminemia (26) and other medical conditions (42). By electrophoresis were highlighted two rare cases of α1-antitrypsin deficiency. Serum protein electrophoresis in children is recommended as a diagnostic technique for increasing the accuracy of the diagnosis in cases of immunodeficiency, liver disease, nephrotic syndrome and acute, subacute and chronic inflammatory diseases.

Keywords: electrophoresis, serum proteins, pathological pattern, children

Human plasma is a complex solution which contains more protein, probably hundreds, some of which are present in insignificant quantities. Serum proteins are represented mainly albumin (Mmol. 69,000 Da), globulins (140,000 Da) and fibrinogen (400,000 Da). Minor fraction is consists of lipoproteins, mucoproteins, antibodies, enzymes and hormones. Serum proteins perform multiple functions in the body, including: the role of carrier, transporting certain ions and molecules (lipids, hormones, vitamins); role in controlling the activity of different proteolytic enzymes; role in regulation of osmotic pressure and buffers [1]. In humans, the albumin is the most abundant plasma protein, it represents 55-60% of serum protein measured [2]. The distribution of these proteins on fractions in healthy people is relatively uniform. However, in certain conditions there are variations in the amount of individual protein components of the plasma (dysproteinemia), rarely meet higher amounts of protein or develop the abnormal protein in response to various conditions. The amount of albumin is regulated by nutritional and hormonal status (insulin, glucagon, cortisol and thyroid hormone), and is synthesized in the liver at a rate of 9-12 g/day [(3, 4]. The decrease in serum albumin level (hypoalbuminemia) occurs in various pathological conditions [5, 6] and has different meanings (malnutrition, malabsorption, nephrotic syndrome, and neoplasia). Deficiency of α1-globulin is associated with lung disease (early onset emphysema) and liver (neonatal hepatitis), lowering the α2-globulin level can be found in acute severe pancreatitis, hepatocellular damage, disseminated intravascular coagulation syndrome, hemolytic anemia, megaloblastic anemia. Serum level of β-globulins in autoimmune disease decreases in active spurt, nephrosis, hepatic diseases, cancer, acute and chronic infections. The percentage of γ-globulins decreases in agamma-globulinemia, hipogammaglobulinemia and nephrotic syndromes [7]. Albumin levels are increased in case of dehydration, and the gammaglobulins in multiple myeloma, rheumatoid arthritis and systemic lupus erythematosus, cirrhosis and acute and chronic infections. The low level of serum albumin is an important prognostic indicator that is associated with increased mortality and morbidity [8, 9]. Electrophoresis on agar, cellulose acetate or polyacrylamide gel is a simple and reliable method of separating protein fractions based on their physical properties (charge, molecular weight and shape of the protein). The technique is based on moving charged particles through a solution when they are subjected to an electric field [10]. Numerous studies have shown the importance of the electrophoretic fractionation of serum proteins in diagnosis of various diseases or disorders where abnormal protein level is found in plasma [11-16]. In Romania there is little information on the subject of serum proteins and their value for the medical practitioner in the management of various diseases in children.

The aim of this study consisted in electrophoretic screening of the levels of serum protein fractions in the children and adolescents hospitalized for various diseases in Emergency Clinical Hospital for children Sfantul Ioan in Galati, and determining the significance of abnormal protein levels in serum.

Experimental part

The study group was represented by 966 patients hospitalized in the Emergency Hospital for Children „Sfantul Joan" in Galati during the period 01.01.2014-01.01.2015 for various diseases, aged between 1-18 years. All subjects included in our study or their legal representatives signed informed consent form. For all the patients have been completed worksheets with the physiological, pathological and demographics data and peripheral blood samples were collected for different laboratory determinations. Patients serum for electrophoretic testes has been separately immediately after blood collection and storage. Serum protein electrophoresis was performed using

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automatic analyzer EXPRIME72, which uses as solid support for separate protein fractions cellulose acetate foils, electrophoresis chamber and alkaline buffer, *p*H 8.9 (*p*H 8.8 Tris buffer and tricine 0.4% diluted 10 times with distilled water); at this pH value, all the protein components are negatively charged and migrate under the influence of electrical current to the positive pole. Sera of patients are loaded into the tray of sample wells and are applied to the cellulose acetate foil with the two metal wires of each applicator. Protein fractions migrate to the positive pole under the action of current with the intensity of 10 mA, for 12 min. Staining was performed with red Ponceau S 0.2% in trichloroacetic acid 3%, which is fixed on protein fractions, and discoloration with destaining solution (citric acid 1 M/L, sodium phosphate bi-basic 1 M/L, and sodium azide <0.02%) diluted 1:20 with distilled water. Following the second stage of washing/bleaching, protein fractions become visible in form of strips. The foil after drying was scanned at *OptiBlue* measuring chamber. On the electrophoregramme, after staining, the proteins appear in the form of bands at that are measures the optical density, each band having a maximum absorption (fi. 1).

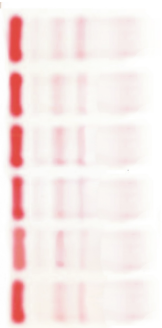

Fig.1. Electrophoretic patterns

Accordingly, five fractions are visualized by electrophoresis: serum albumin, α1-, α2-, β- and γ-globulins. Albumin, α1- and β-globulins appear as homogeneous bands, well defined. α2- and γ-Globulins appear as diffuse bands, γ fraction showing in its central part a more intensely colored area. Reference values for separate protein fractions on electrophoregramme dependent on age are shown in table 1. Interpretation of electrophoretic serum protein patterns was done according to the guide of Laboratory Corporation of America, Directory of Services and Interpretive Guide, Protein Electrophoresis, Serum, 2011 [17].

Total serum proteins were determined by biuret reaction, using the biochemical automatic analyzer VITROS 950 and reagents supplied by Ortho Clinical Diagnostics, Johnson-Johnson Company, UK. The proteins react with the cupric ion of copper tartrate in an alkaline medium, giving a purple complex. The extinction of the colored compound obtained is measured spectrophotometrically at λ = 540 nm and is proportional to the amount of total protein in serum, expressed in g/dL. Electrophoretic results and total protein were analyzed in correlation with the age of the patients.

The data were analyzed using SP SS version 20 for windows software package. Statistical procedures used to analyze the data included Chi- square test, T-test, ANOVA and Pearson correlation. Quantitative variables are presented as mean ± SD and qualitative variables as percent. Differences between groups were considered significant when $p < 0.05$.

Results and discussions

This study included 966 pediatric patients with mean age of (6.47 ±5.33) years, of which 484 (average age of 50.1%; 6.29 ± 5.17 years) were boys and 482 (49.9%; 6.60 ± 5.50 years) girls, the difference between sex not being statistically significant. Pediatric patients were divided into 3 age groups: < 1 year, between (1-16) years and > 16 years. The distribution of age groups indicated in figure 2 show a higher prevalence of children aged (1-16) years, for both boys and girls.

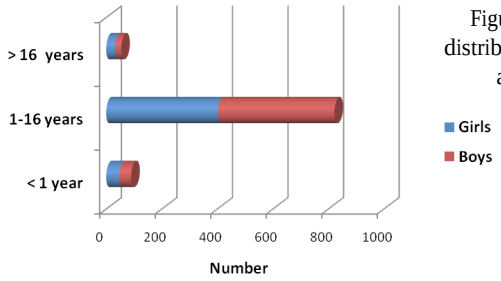

Figure. 2. Lot distribution by age and sex

The results concerning at clinical and paraclinical parameters, presented in table 2 indicate the wide variation limits for each parameter was analyzed. A number of 333 patients (31%) have presented abnormality electrophoretic patterns, serum levels of total protein, albumin and globulins were not framed in reference ranges recommended for children (table 1). Serum total protein losses correspond mainly to a reduction in the levels of albumin, less being influenced by the decrease or increase in globulins. The decrease in serum albumin levels were recorded in 50 cases (5.2%) and the increase at 2 patients (0.2%), in comparison with gamma globulin levels which were elevated in 33 cases (3.4%) and lower in the 77 cases (8%).

Comparative analysis of clinical and paraclinical (TP, A, G α1, α2-G, β and γ-G) parameters, depending on age group and diagnosis at admission, indicates statistically significant differences for all analyzed parameters ($p < 0.05$). Age was significantly correlated with total protein

Parameters	Age (Years)		
	< 1	1-16	>16
Serum total protein (PT), g/dL	4.6-7.3	6.0-8.0	6.6-8.7
Albumin (A), g/dL (%)	1.84-4.74 (40-65)	3.00-5.20 (50- 65)	3.43-5.91 (52-68)
α1-Globulin (α1 - G), (g/dL) (%)	0.09-0.36(2-5)	0.13-0.43 (2-5)	0.13-0.43 (2-5)
α2-Globulin (α2 - G), g/dL (%)	0.30-0.98 (6.6- 3.5)	0.36-1.2(6-15)	0.43-1.17 (6.6-13.5)
β-Globulin (β – G), g/dL (%)	0.39-1.02 (8.5-14)	0.51-1.2 (8.5-15)	0.56-1.21(8.5-14)
γ-globulin (γ – G), g/dL(%)	0.46-1.53 (10-21)	0.66-1.76 (10-22)	0.72-1.82 (11-21)
Albumin/Globulin report (A/G)	1.16-2.23	1.06-2.48	1.39-2.23

Table 1
REFERENCE RANGES FOR TOTAL PROTEIN AND PROTEIN FRACTIONS ISOLATED THROUGH ELECTROPHORESIS, BY AGE GROUPS

(r=0.466; p< 0.001), albumin(r=0.188; p< 0.001), α2 – globulin (r=- 0.107; p= 0.001), β -globulin(r=0.438; p< 0.001) and γ - globuline (r=0.579; p< 0.001).

On admission, children had different symptomatologies. The distribution of serum protein fractions according to the electrophoretic appearance is shown in table 3. Most patients have been showing subacute inflammatory response (12.5%), followed by hipogammaglobulinemia (6.9%), acute inflammatory response (3.3%), polyclonal hipergammaglobulinemia (2.5%), and chronic inflammatory response (1.1%); for the rest of diseases were registered percentages under 1%.

In figures 3 a, b, c, d are shown examples of electrophoretic patterns healthy patients, with syndrome nephrotic and acute and chronic inflammatory response.

Normal constituents of blood plasma have electrophoretic pattern fairly uniform at healthy people, although slight variations are possible due to genetics and diets. These patterns are altered in some diseases and nutritional disorders. The changes manifest themselves, in general, by increasing or decreasing the levels of proteins, which are distinguished by electrophoresis. The decrease in the concentration of serum proteins, especially albumin, is the primary consequence of reducing the rate of synthesis, inflammation being known to cause suppression of albumin transcription gene. Also, protein malnutrition can affect a patient's protein synthesis. Loss of plasma proteins contribute to decrease of serum proteins levels, through crossing hemodialysis membranes [18].

In children with nephrotic syndrome (0.3%), we found low levels of albumin (2.36 g/dL), relatively normal at α1-, β- and γ-globulins and increased in the case of α2-globulin (fig. 3 b). Our results confirm those reported by the Volturi et.al. [19], which he recorded in children with nephrotic syndrome by albumin levels of 2.0 ± 0.55 g/dL. Nephrotic syndrome is a consequence of alteration the selective permeability of glomerular membrane, which leads to the loss of albumin and other serum proteins with intermediate molecular weight and at increase the concentration of molecules with large molecular mass [20]. Nephrotic syndrome is characterized by proteinuria >3.5 g/24 h, edema, hypoalbuminemia and dyslipidemia. Etiological causes of nephrotic syndrome vary from primary renal disease to systemic disease with presentation different histopathology [21, 19].

We found a similar situation and in the case of children with serum protein losing enteropathy (0.7%), but electrophoretic parameter values were slightly elevated compared with nephrotic syndrome, with exception α2-gamma globulin, for which was found a lower level. Protein losing enteropathy is a disorder characterized by an abnormal loss of serum proteins in the digestive tract or its inability to absorb proteins [22, 23]. It is an atypical clinical condition that can appear in the context of the other diseases caused by bacterial or parasitic infections, celiac disease, Crohn's disease, lymphoma, HIV infection or after surgical correction of congenital heart disease. The increase losses to plasmatic protein occur due to damage of intestinal mucosa with or without erosion/ulceration (Schmidt, et al., 1995). Respective authors have found losses of serum albumin and immunoglobulin G (IgG) in faeces at 13% patients. Protein losing enteropathy meets up to 10% patients due to complications of the Fontan operation with a very high mortality rate [24].

Slight imbalances between synthesis and metabolism of proteins and protein losses have been recorded by us and in cases diseases with acute, subacute and chronic inflammatory response (16.9% cases). Mean values for albumin levels ranged in range (3.43-3.52) g/dL, being close to the lower limit of the reference, and globulins were

Parameters	No.	Mean	Minim	Maxim	SD*
Age (years)	966	6.47	0.00	18.00	5.33
Total serum protein (TP), g/dL	966	6.92	3.50	9.30	0.76
Albumin (A), g/dL	966	3.64	0.63	5.48	0.45
α1-globulin (α1-G), g/dL	966	0.25	0.07	0.62	0.06
α2-globulin (α2-G), g/dL	966	1.01	0.40	1.73	0.18
β- globulin (ß-G), g/dL	966	0.90	0.37	1.51	0.16
γ-globulin (γ-G), g/dL	966	1.10	0.18	2.49	0.36
Report Albumin/Globulin (A/G)	966	1.14	0.22	2.32	0.25

*SD - Standard deviation

Table 2
CLINICAL AND PARACLINICAL PARAMETERS OF THE SUBJECTS IN STUDY GROUP

Electrophoretic pattern	No. (%)*	Albumin (g/dL)	α1-globulin (g/dL)	α1-globulin (g/dL)	β- globulin (g/dL)	γ-globulin (g/dL)
Protein losing enteropathy	7 (0.7)	2.70±0.43	0.45±0.10	1.11±0.16	0.96±0.27	1.34±0.64
Nephrotic syndrome	3 (0.3)	2.36±0.34	0.24±0.13	1.37±0.32	0.73±0.22	0.54±0.15
Hipogammaglobulinemia	67 (6.9)	3.61±0.45	0.20±0.05	0.90±0.18	0.73±0.14	0.41±0.15
Polyclonal hipergammaglobulinemia	24 (2.5)	3.47±0.66	0.27±0.05	0.94±0.18	0.92±0.16	1.84±0.49
Chronic inflammatory response	11 (1.1)	3.84±0.47	0.27±0.06	1.19±0.19	1.25±0.09	1.76±0.38
Acute inflammatory response	32 (3.3)	3.43±0.47	0.36±0.11	1.16±0.24	1.10±0.23	1.20±0.43
Subacute inflammatory response	121 (12.5)	3.52±0.46	0.28±0.06	1.29±0.12	0.94±0.15	1.10±0.32
Hypo α1-globulinemia	2 (0.2)	4.01±0.09	0.08±0.01	0.66±0.36	0.76±0.45	1.43±0.39
Hypoalbuminemia	26 (2.7)	2.87±0.28	0.25±0.05	0.91±0.17	0.86±0.18	1.26±0.34
Other conditions	40 (4.1)	-	-	-	-	-

* Percentages reported by the total number of patients (966)

Table 3
ELECTROPHORETIC PATHOLOGICAL PATTERN IN STUDY GROUP

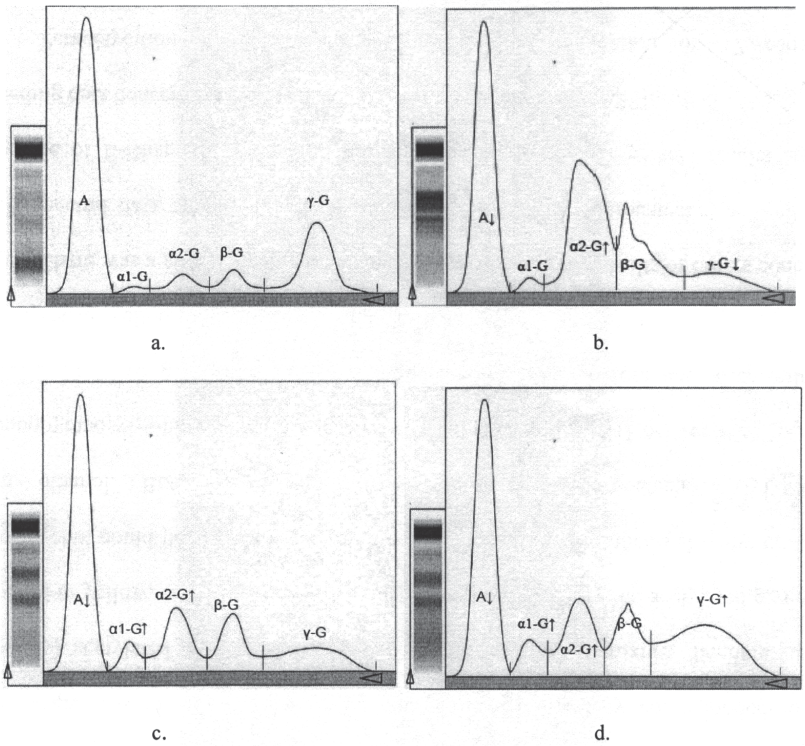

Fig. 3. Electrophoretic patterns: a. normal; b. nephrotic syndrome; c. acute inflammatory response; d. chronic inflammatory response

situated closer to the maximum level (table 3, figs. 3 c, d). Patients with chronic inflammation had higher levels of serum γ-globulin. Inflammation is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants [25]; protective response involved immune cells, blood vessels and molecular mediators.

On the basis of electrophoretic pattern: albumin level decreased ; the values for α1-, α2- and β-globulin normal; level of γ-globulin increased and the lack of other symptoms, 24 (2.5%) children were diagnose with polyclonal hypergammaglobulinemia. Hypergammaglobulinemia is due to activation of a large number of plasma cell clones that release immunoglobulins. Increases of polyclonal gamma globulin at the adult indicates an immunological process associated with chronic liver disease (chronic active hepatitis, cirrhosis), collagen diseases, cancers, leukemia, chronic myelomonocytic. Polyclonal gammopathy is associated with diagnosis of autoimmune diseases [26, 27]. The highest level of γ-globulin was recorded in girls (2.49 g/dL), compared to 2.19 g/dL in boys. Lo, et.al. [27] suggest that girls with high levels of IgG are three times more susceptible to autoimmune disorders than boys. Levels range (1.90-2.49) g/dL were found in children with gastroenteritis, liver damage, nonspecific fever, chronic bronchitis, digestive disorders and functional abdominal pain, sepsis, arthritis, protein losing enteropathy. Other authors have found high levels of gamma globulins in the case of autoimmune or autoinflammatory diseases, bacterial infections, liver diseases, syndromes associated with drug treatment, tumors/lymphoproliferative, hematopoietic stem cell transplantation, primary immunodeficiency [27].

Hypogammaglobulinemia is a type of primary immune deficiency, and consists of abnormally low concentrations of blood gamma globulins [7]; particularly IgG as the primary antibody from the circulatory system and immunoglobulin A (IgA). Hypogammaglobulinemia was present in 67 (6.9%) children with average age of 1.6 ± 2.24 years for which the average gamma globulin level were of 0.18 g/dL, value below the minimum limit indicated for the age those children. It is possible that these children to submit transient hypogammaglobulinemia when IgG and IgA levels can remain low in the first 6 months after birth, either are not yet produced or the formation of antibodies is prevented because of malnutrition. Gamma globulin deficiency has been associated with various disorders, such as gastroenteritis, intestinal bacterial and urinary tract infections, disorders of digestive function, nonspecific fever, allergies, liver damage, nonspecific jaundice, malnutrition and other diseases. Most children with hypogammaglobulinemia are presented with history of recurrent infection, mainly with primary immunodeficiency [28, 29].

Hypoalbuminemia, medical condition in which blood albumin levels are abnormally low, was present in 26 (2.7%) patients with mean age of 10.04 ± 6.68 years. Serum albumin values were within the range (2.15-3.42) g/dL, levels considered to be low (<3.5 g/dL). These patients benefit from albumin infusion for a significant increase in the level of albumin, mechanical ventilation due to respiratory insufficiency and prolonged residence in the hospital. The disease can progress to multiorgan dysfunction syndrome and increases the risk of mortality. Hypoalbuminemia in most cases correlate with intestinal protein loss that is associated with pneumonia with pleural effusion in children [30]. It is considered a significant indicator of mortality and morbidity in critically sick children [31, 32].

We have found electrophoretic pattern specific condition hypo α1-globulenimia: normal levels of albumin, α2-, β- and γ-globulins and low level (0.07-0.09) g/dL of α1-globulin (α1-antitrypsin) only at 2 patients (0.2%) with mean age of 3.5±4.95 years. In America, less than 10% persons are recognized with clinical levels of α1-antitripsin smaller value threshold of protection 11 μM [33, 34] or less than 0.21 g/dL [35] α1-Antitrypsin deficiency is a genetic disorder due to lower production and decreasing the activity of the protein in the blood. Hypo α1-globulinemia leads in early onset emphysema and liver disease.

Serum protein electrophoresis, although not is itself a diagnostic test, offers information that help at disease diagnosis more accurately. Increase and decrease levels

of the five major groups of proteins in the bloodstream indicates there the electrophoretic evidences of some major health problem: malnutrition, immunodeficiency, nephrotic syndrome, dehydration, acute infections, chronic inflammatory disease, liver disease, multiple myeloma, macroglobulinemia and hyperimmunization.

Conclusions

Serum protein electrophoresis is a screening test that separate, determines and analyzed of major fractions protein levels in the plasma, with important roles in many biological responses.

Serum protein electrophoresis helps pediatrician to diagnose, assess and monitor the disease course in patients with renal and intestinal protein loss, immune disorders, hepatic dysfunction, insufficiency nutrition, inflammatory conditions and critical disease (cancer).

Electrophoretic screening of serum protein should be a routine laboratory test applied to all children at admission; he can provide valuable clues about the location, cause and severity of the disease.

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