Clinical and paraclinical aspects in mental retardation and autism spectrum disorders

Retardul mental şi tulburările din spectrul autist – aspecte clinice şi paraclinice

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Abstract

Mental retardation (RM) and autism spectrum disorders (ASD) are mental illnesses that severely impair individual development and have serious consequences that affect both family and community. The frequency of such illnesses is increasing, calling for a unitary collective effort towards diagnose and treatment. The study involved 62 patients diagnosed with RM and/or ASD, associated with anemia, hearing or sight impairments, epilepsy and also 14 cases of viral hepatitis type B. The study aimed to corroborate clinical information with paraclinical evidence in order to survey individual patient health evolution. Tests showed that patients with intellectual disabilities, whether receiving neuroleptic treatment or not, are frequently afflicted by anemia and water imbalances and very often exhibit metabolic acidosis. Such identified metabolic abnormalities should be justified by genetic and/or environmental factors. The complexity of autism spectrum disorders etiology invokes the multifactorial character of these diseases.

Keywords: mental retardation, autism, anemia, metabolic acidosis.

Rezumat

Retardul mental (RM) şi tulburările de spectru autist (TSA) sunt boli mentale care afectează grav dezvoltarea individuală, având consecinţe deosebite în plan familial şi social. Frecvenţa acestor deficienţe este în creştere, necesitând un efort multidisciplinar cumulat şi unitar de diagnosticare şi tratament. Studiul a fost efectuat pe un lot de 62 de pacienţi cu diagnostice de RM şi/sau TSA asociate cu anemii, deficienţe de auz şi de văz, epilepsie, 14 pacienţi având şi complicaţii cu hepatită virală de tip B. Studiul a avut ca obiectiv corelarea infor-

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maţiei clinice cu dovezile paraclinice în scopul evaluării evoluţiei stării de sănătate a pacienţilor. Testele efectuate au demonstrat că pacienţii cu dizabilităţi intelectuale, cu sau fără tratament neuroleptic, sunt afectaţi frecvent de anemii şi dezechilibre hidrice, manifestând frecvente acidoze metabolice. Modificările metabolice sesizate trebuie justificate prin influenţa factorilor genetici şi/ sau de mediu. Complexitatea etiologiei tulburărilor de spectru autist susţine caracterul multifactorial al acestor afecţiuni.

Cuvinte cheie: *retard mental, autism, anemie, acidoză metabolică.*

Introduction

Mental illnesses require special attention in human pathology both because of their frequency and of their issuing social impact, involving both family and community over long term periods. Among these, autism spectrum disorders (ASD) [1,2] call all the attention of the medical community because of their prevalence and ever-increasing incidence. Although the estimation of prevalence of autism and ASD is controversial because of the differences between identification methods [3,4,5,6,7], the last years have witnessed a considerable growth of autism prevalence throughout the world [8,9,10,11,12]. According to European specialists, actual frequencies were estimated at the International "Autism Europe" Congress (Lisbon, 2003) at 10-40 /10,000 for infantile autism and at 60-70 /10,000 for ASD, approximately 10 times higher than pre-1990 estimates. The growth rate of worldwide occurrences has attained the alarming value of 3.8% per year, with a sex ratio – boys to girls – of 3:1. In the United States, autism has become the fastest-growing developmental disability, with an annual 10-17% growth predicted by the Network of Autism Training and Technical Assistance Programs (NATTAP) and the Autism Society of America (ASA) at the Second Annual International Autism Training and Technical Assistance Conference (Ohio, 2008).

According to growing frequencies in the last few years, we can estimate that 6 million Romanian children (according to the National Authority for Child Welfare and Adoption, May 2001) about 6000 suffer from classic autism and 36,000 to 42,000 from ASD.

There are large areas of interference between mental retardation (MR) and autism spectrum disorders. Around 50-75% of autistic children can be defined as mentally retarded. Recent data suggest that the increase in cases of autism corresponds to a decrease in cases of mental retardation [10]. Changes in how both autism and mental retardation are classified could cause an artificial increase in autism cases. It is possible that children with both mental retardation and autism could be classified as having mental retardation with autistic features. On the other hand, ASD patients over 18 years of age receive diagnoses of mental retardation or schizophrenia, as international classifications of mental illnesses limit pervasive development disorders to childhood.

Mental retardation prevalence was estimated at around 3% of general population, higher in developing countries, as a result of more frequent accidents and oxygen deprivation in pre- and postnatal periods, as well as childhood cerebral infections [13,14,15]. More recent studies estimate that mild mental retardation represents around 85% of all MR cases and occurs in 20-30 out of 1000 people in the general population, while severe mental retardation occurs only in 3 or 4 out of every thousand. Both forms affect preferentially men, with a 40-80% excess rate (sex ratio men: women of 1.4-1.8) for mild MR and a 20% excess rate (men: women of 1.2) for severe MR [16,17]. On the whole, occurrence sex ratio stands at 1.5 (men: woman). A large spectrum of diseases can also be associated with mental disorders, like epilepsy [18], sight and hearing deficiencies or language and expression impairments. The more illnesses associated, the more severe the MR.

Table 1. Study group composition by diagnosis, age and sex

Diagnosis	Female		Male	
	Under 18 yrs. old	18-22 yrs. old	Under 18 yrs. old	18-22 yrs. old
MR	4	7	11	17
MR+ASD	4	2	5	4
ASD	2	0	6	0

Table 2. Study group composition by caretakers and educational level

Diagnosis	Family		Institution	
	Trainable	Untrainable	Trainable	Untrainable
MR	0	5	0	34
MR-ASD	3	1	2	9
ASD	3	4	1	0

ASD and MR are multifactorial diseases that combine both genetic and environmental factors, the manifestations of which can be identified by the use of various laboratory tests [19,20,21,22,23]. Data provided by the clinical lab aim to portray the current level of affliction, corroborating patient history and suggesting directions for treatment evolution, where this is due.

Materials and methods

The study was carried out on 62 patients with initial diagnoses of MR and ASD, either simple or associated. All diagnoses were established by qualified medical personnel. The study group was divided into three subgroups, according to diagnoses, thus: an MR group, an MR-ASD group, and an ASD group. Data regarding study groups' composition is given in *Table 1*.

Considering the severity of intelectual development impairment, expressed by means of IQ, the study group comprised 38 cases of severe mental retardation (IQ = 20-34) and 6 cases of moderate mental retardation (IQ = 35-49). While the other 19 patients had no specified IQ, they fell well, by means of observation, inside one of the aforementioned categories. Most patients belong to the urban environment, 46 of them being institutionalised (*Figure 1*). As concerning educational levels [24], less than a fifth of investigated children are at all trainable and receive normal or specialized education (*Table 2*).

Two thirds of study patients (the institutionalized) have unknown illness history, but, out of the remaining third, 8 ASD and 2 RM patients had hypoxia at birth, 5 ASD children
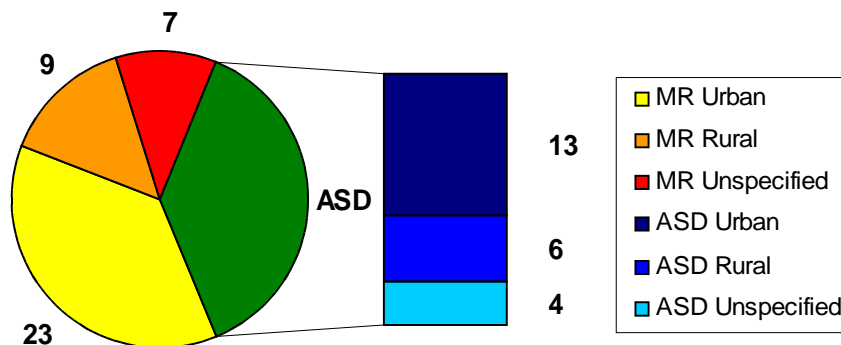
Figure 1. Group composition by social environment

had postnatal anemia and 8 MR patients have congenital malformations (4 concerning the bones and 4 concerning the eyes). In subjects with single or otherwise associated forms of autism there are also cases of eye afflictions (2), skin conditions (2), suspected deafness (4), allergies (2), hepatitis (6) and epilepsy (4). The MR group experienced additional diagnoses of anemia (2), body hypotrophy (4), allergies (2), functional colonopathy (2), gastroesophageal reflux (1), hepatitis (8) and epilepsy (7). Feeding these patients is difficult, nourishment has to be liquefied or very finely ground, as most patients are unable to masticate. Some MR subjects experience an insatiable appetite associated with alimentary reflux or incomplete digestion, while ASD±MR children exhibited alimentary preferences and one case of pica.

As concerning behavior, patients manifest manias or agitation sometimes accompanied by auto- or heteroaggression, and 2 are hyperkinetic. Over 40 patients receive neuroleptic antipsychotic medication (Rispen, Torendo, Levomepromazin, Plegomazin, Haloperidol), anticonvulsants (Orfiril, Carbamazepine, Depakine, Taver, Rivotril, Lamictal, Fenobarbital) and anxiolytic drugs (Diazepam, Nitrazepam, Calmepam, Bromazepam, Meprobamat), to which are added, if needed, liver protectors and anti-ulcer medication (Nexium, Sargenor, Silimarin, Omeprazol) or stimulators (Bilobil, Tanakan, Encephabol, Milgamma, Vi Sol, Ferrum Haussman).

After caretakers signed an informed consent approved by the Ethics Commission of the University "Dunarea de Jos" of Galati, 3 blood samples were drawn from the patients: one was drawn on K_3EDTA, one on heparin and one of total blood. Tests were performed to characterize anemia (complete hemograms, sideremia, total iron binding capacity – TIBC, transferrin saturation), functional hepatic tests (hepatic enzymes activity, serum protein electrophoresis), tests concerning electrolyte levels, muscle enzymes and metabolic products (lactate dehydrogenase – LDH, creatin phosphokinase – CK, glucose, lact-

ate,) as well as serum nitrogenous components, (uric acid, creatinine, urea and ammonia).

All tests were carried out using automated analyzers: *Vitros 950* for tests using Slide method [25], *Exprime72* for serum protein electrophoresis, *Celltak MEK 6400K* for hematological tests.

Result interpretation was performed, after data was corrected with standard deviation, according to reference intervals for biological values as given in literature [26, 27], estimation precision having a confidence interval of 95%.

Results

Hematological investigation results

Analysis showed a decrease in hemoglobin levels below biological references in 29 subjects (20/MR, 6/MR+ASD and 3/ASD). Two cases of anemia, with hemoglobin levels below 10 g/dL, were signaled, both in MR patients. Drops were registered in MCH (mean corpuscular hemoglobin) levels in 10 patients (6/MR, 2/MR+ASD, 2/ASD) and MCV (mean corpuscular volume) levels in 6 patients (4/MR and 2/ASD). Only in one MR subject was the MCV level above normal values. Serum iron levels in study children were between 20-200 µg/dL, with 9 cases below lower biological reference interval (7/MR, 1/MR+ASD and 1/ASD) and 7 cases above upper biological limits (3/MR, 3/MR+ASD and 1/ASD). In patients aged 18 and above, iron levels were between 11-185 µg/dL, with 6 instances of levels below inferior biological limits, all in MR group. TIBC results were all within biological reference intervals (240-480 µg/dL), except for one case in MR group (TIBC = 571 µg/dL). Although all TIBC levels were within normal ranges, transferrin saturation calculations (*%Sat = (Fe / CTLF) × 100*) revealed an iron deficiency in 15 of the patients (13/MR, 1/MR+ASD and 1/ASD). Study group distribution of registered anomalies in the anemia tests is shown in *Figure 2*.
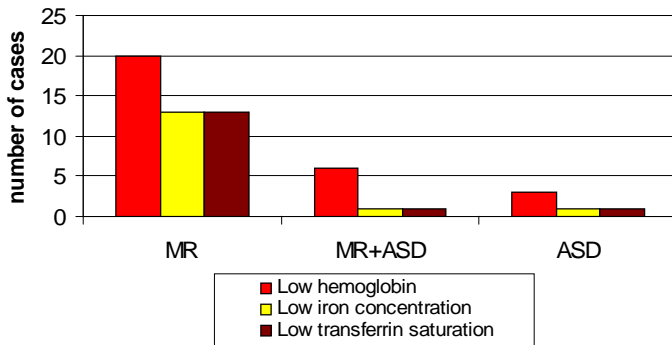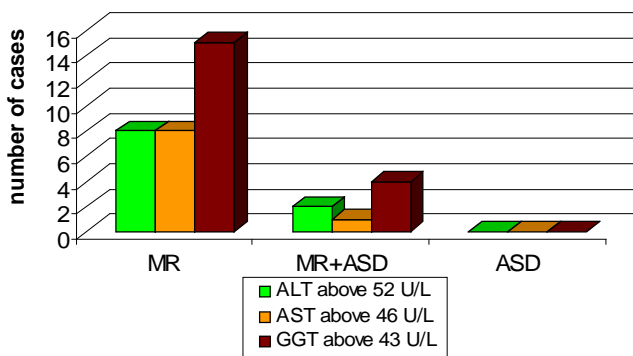
Figure 2. Anomalies in anemia tests

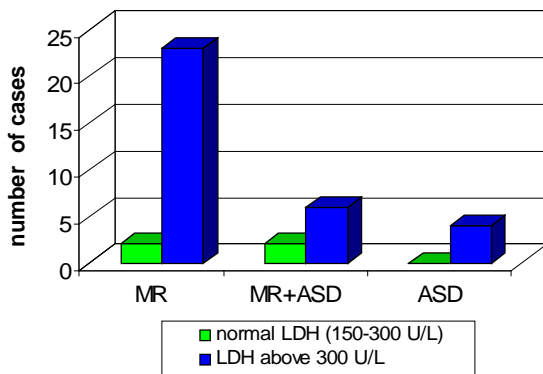**Figure 3. Increased activity of ALT, AST, GGT**

Figure 4. Enzymatic activity of LDH

In 54 patients, leukocyte levels were within normal ranges for the age group. There were 8 instances of leukocytosis (4/MR, 2/MR+ASD and 2/ASD). Only one patient in the MR group exhibited a white blood cell count slightly below reference ranges. In 15 pa-

tients, white blood cell count summary showed increases in lymphocytes, granulocytes and eosinophils, 7 out of these cases occurring without leukocytosis. There were 5 cases of granulocytosis (1/ASD and 4/MR, one of which was associated with lymphocytosis), 10 lymphocytoses, (2/ASD, 4/MR+ASD, one of which associated with eosinophilia, 4/MR, two of which associated with raised granulocytes or eosinophilia) and 3 cases of eosinophilia (1/MR-ASD, 40% eosinophylia and 2/MR, with levels at 11% and 25%). By referring to normal biological ranges (150.000-350.000 thrombocytes/μL), we identify 4 thrombocytopenias (2/MR and 2/MR + ASD) and 16 thrombocytoses (11/MR, 2/MR+ASD and 3/ASD). Erythrocyte sedimentation rate (ESR) was normal with the exception of 11 abnormal increases in MR patients and 2 in MR-ASD patients.

Functional hepatic test results

High levels of hepatic enzymes ALT (alanine aminotransferase), AST (aspartate aminotransferase) and GGT (gamma-glutamyl transpeptidase) were registered in 31 MR patients and 7 MR+ASD patients (*Figure 3*). Lactate dehydrogenase activity revealed elevated levels in 33 patients, as shown in *Figure 4*. AST, ALT and LDH levels were raised by 1.5 to 2.5 times and GGT levels were up 1.5 to 6 times. In 4 patients infected with hepatitis B simultaneous increases were registered in AST, ALT, GGT and LDH enzymatic activity. Alkaline phosphatase activity was high in 5 subjects (4/MR and 1/MR-ASD). Serum proteins were within normal ranges, in all but 10 patients (5/MR and 5/MR+ASD), who displayed elevated protein levels, between 8.4 and 9.8 g/dL. Serum protein increases were evident in relevant electrophoretic fraction modifications (albumins, alfa-2 and gamma-globulins). Serum protein electrophoresis revealed raised protein

Table 3. Electrolyte imbalance

mmol/L No. of cases	Na$^+$ above 145 mmol/L	K$^+$ above 5 mmol/L	Ca^{2+} below 1.18 mmol/L	Mg above 0.94 mmol/L	ECO$_2$ below age re- quirements	Cl$^-$ above 108 mmol/L	Phosphates above age requirements
MR	36	7	39	0	0	6	10
MR+ASD	10	5	15	0	0	0	5
ASD	5	4	8	1	1	5	4

fraction levels even when total protein concentrations were normal, for example: 23 cases of elevated albumins (14/MR, 3/MR+ASD, 6/ASD), 16 cases of elevated gamma-globulins (13/MR, 3/MR+ASD) and 16 cases of elevated alfa-2-globulins (10/MR, 3/MR+ASD, 3/ASD). Albumin/globulin ratios were subunitary in 8 subjects (5/MR and 3/MR+ASD), 4 of which infected with hepatitis B virus.
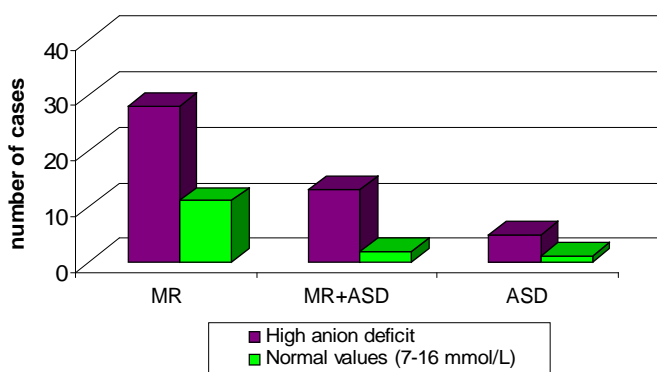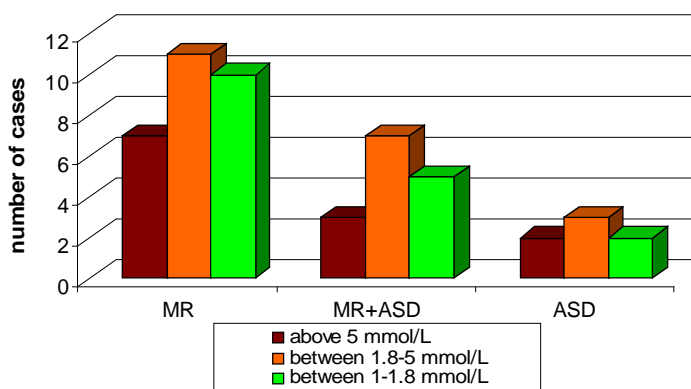

Figure 5. Anion gap

Figure 6. Lactate concentrations

Serum ion concentration test results

Abnormalities concerning ions involved in acid-base equilibrium, sorted by study group, are listed in *Table 3*.

According to test results, total blood calcium levels were above normal ranges in 9 patients (5/MR, 1/MR+ASD, 3/ASD) and below those in one MR patient. Ionic calcium concentration, calculated from the McLean-Hastings nomogram, was below reference inferior normal levels in all investigated patients.

Anion deficit (anion gap) is calculated using the formula: *Anion deficit = Na$^+$ - (ECO$_2$ +Cl$^-$)*, and reveal a high level of metabolic acidosis, which occurs in 28 MR patients, 13 MR+ASD and 5 ASD patients (*Figure 5*).

Muscle activity investigation results

Test showed slightly lowered blood glucose levels in 6 subjects (4/MR and 2/MR+ASD). Lactate doses showed elevated levels in 33 patients, as follows: in 21 cases (11/MR, 7/MR+ASD, 3/ASD) lactate levels were between 1.8 – 5 mmol/L, while in 12 patients (7/MR, 3/MR+ASD, 2/ASD) lactate was above 5 mmol/L and up to 10 mmol/L (*Figure 6*). Four patients also registered increases in CK activity (3/MR and 1/MR+ASD).

Serum nitrogenous components level test results

Blood urea levels were elevated for 23 subjects (10/MR, 6/MR+ASD, 7/ASD), all aged below 19. Creatinine

concentration was 0.1 mg/dL lower than normal in 37 cases (28/MR and 9/MR+ASD), the rest of the group registering normal. Uric acid levels were normal in all but 5 cases (1/MR, 3/MR+ASD, 1/ASD) which registered below normal ranges. Serum ammonia levels were increased by as much as 20% from average ranges (11-48 µmol/L for girls and 15-55 µmol/L for boys) in 3 MR patients, 2 MR-ASD and 1 ASD patient. Three of these cases had ammonia levels above 60 µmol/L.

Measurements for sodium, urea and glucose concentrations served to calculate osmolality (CO), using the formula: $CO = 2Na^+ + Glucose/18 + Urea/2.8$. Reporting to a reference range of 275-295 mOsm/kg, raised osmolalities were registered in 14 of the investigated patients (4/8ASD, 6/15 RM+ASD and 6/39MR).

Discussions

Low levels of hemoglobin, MCH, MCV, iron and transferrin saturation revealed 33 cases of anemia (22/MR 6/MR+ASD and 5/ASD). Ten patients were diagnosed with hypochromic anemia, 6 of which were cases of microcytic anemia. Fifteen patients were diagnosed with iron deficit anemia, confirmed by blood iron measurements and transferrin saturation calculation. Patients receiving treatment for pre-diagnosed anemia syndromes registered normal for iron and transferrin saturation. By reuniting the two categories, the total number of patients with anemia exceeds one half of investigated subjects, with ferriprive anemia present in almost one fourth on the study lot. One case of macrocytic anemia was identified in one MR patient, who also exhibited gastroesophageal reflux disease and alimentary atresia – which would justify folic acid/B12 deficits. As for the rest of the patients exhibiting low hemoglobin, anemia is associated with functional hepatic deficiencies (coupled with raised ALT, AST and GGT), and accompanies inflammatory phenomena, justified by elevated ESR and electrophor-

etic fractions α1 and/or α2 or is caused by impaired nourishment.

Anemia frequency is elevated in male investigated subjects (*sex ratio* boys: girls 2:1) and in the institutionalized (30/46 institutionalized patients), in which the deficit will be maintained beyond childhood. Ten institutionalized patients with anemia also suffer from chronic hepatitis B. Children with ASD are pale and lanky, never overweight, as a result of preferential nourishment, hyperactivity and sleep disorders.

Results are in agreement with literature in pointing to iron deficits of cerebral hypoxia as causes for incomplete mental development and as negative influences on cognitive and affective functions [28,29]. Anemia in MR and ASD may be connected to pre-, peri- or postnatal events corresponding to maternal health issues (malnutrition, anemia, viral or bacterial infections that pass through the placenta), to birthing conditions (premature delivery, premature clamping of the umbilical cord, accidents and oxygen deprivation) or to environmental conditions in early life (infections, intoxications, malnutrition) [30]. Anemia may be a lifelong one, carried on by nutritional deficits – since ASD children often exhibit strict preferences for food – or by insufficient nutrient absorption due to inadequate mastication or to digestive/chronic diseases already present. A study by E. Hurtado [31] regarding associations between anemia and intellectual retardation claims that the risk of light or moderate mental retardation increases 1.28 times with decreasing hemoglobin.

Another significant aspect revealed by this study is the presence of acidosis in 46 of the investigated subjects. Acidosis, a perturbation in blood pH (normally 7,32 – 7,45), implies an excess of acid in the blood stream, whether bicarbonate or endogenous/exogenous organic acids [32]. Although the present study did not determine blood pH level and O_2/CO_2 pressures, frequent high anion gaps associated with hypernatremias, despite hyperphosphatemias

Table 4. Differences between partially compensated metabolic acidosis and acidosis in MR and ASD

Partially compensated metabolic acidosis	$CO_2\downarrow$	$Na^+\downarrow$	K^+	Cl^-	Osmolality↑	proteins	Hb↑
Difference:	**WATER**						
Metabolic acidosis in MR and ASD	CO_2	$Na^+\uparrow$	$K^+\uparrow$	$Cl^-\uparrow$	Osmolality↑	proteins↑	Hb↓

and a few hiperchloremias, with normal potassium and magnesia levels, with normal bicarbonate concentrations and insignificant hyperproteinemia, show the inefficiency of blood acid-base buffering and change electrolyte balance into acidosis. Sodium concentration correlates well with anion gaps (correlation coefficient 0.67/MR, 0.84/MR+ASD, 0.72/ASD) and strongly with osmolality (correlation coefficient 0.86/MR, 0.87/MR+ASD, 0.99/ASD) in all study groups.

Elevated ammonia, reported in 6 patients, also contributes to the increase of the anion gap. Differentiation between the types of acidosis is achieved by means of increases in lactate concentration. Out of 33 registered cases of metabolic acidosis, 12 progressed to lactic acidosis. Metabolic acidosis in investigated subjects is partially compensated. It should be noted, however, that MR and ASD patients have a particular biological profile for partially compensated metabolic acidosis (*Table 4*).

The comparison between the two biological profiles reveals a reduced amount of water in study patients' blood. Should hydration be normal, carbon dioxide levels would decrease and potassium, chloride and sodium would normalize or drop below reference, and so would proteins. Normal water contents would also lead to normalization of blood cell number, decreasing hypercellularity, leaving only the cases of cellular deficit and the 3 eosionophilias. On the other hand, normal hydration would only accentuate the frequent anemia, by further diluting hemoglobin and thus aggravating tissue hypoxia. Hemoglobin-oxihemoglobin buffer is ineffective in either case.

A correction in hydration balances might also reduce phosphate levels towards normal, which in turn would determine a drastic decrease in calcium. Since high electrolyte concentrations in extracellular space induce water flow from the inside of the cell, with intracellular dehydration as result, and considering that extracellular space is marked by severe hypernatremia in over 50 patients, cell content should be correspondingly high in potassium or calcium ions. Constant increased serum ionic concentrations suggest alterations in active transport through ionic channels or water metabolism.

A normal water balance implies that a daily intake of 0.5-5 L of water corresponds to a renal excretion of 0.5 – 15 ml/min (approx. 0.5 - 4 L/day) together with a further 500 – 850 ml lost through perspiration and respiration. Pathological water loss is correlated with fever, digestive illness manifested by vomiting and diarrhea or excretion pathologies. Renal water elimination is controlled by arginine-vasopressin, subsequently referred to as an antidiuretic hormone. Differences between extra- and intracellular osmolality, perceived by the hypothalamus, are manifested by adjustments in arginine-vasopressin secretion in the posterior hypophysis. Increases in osmolality in a healthy organism lead to a secretion of antidiuretic hormone and increased water retention at the kidneys [33]. Also, extracellular volume modifications influence aldosterone secretion by the adrenal gland, resulting in a modulation of urine sodium. Furthermore, the kidney itself adapts to compensate acidosis by increasing ammonia production. The study lot contained few cases of hydric imbalance of gastro-intestinal causes

and no cases of fever. Although there were some occurrences of elevated ammonia, serum nitrogenous components tests are insufficient to characterize the quality of excretion and therefore further 24-hour urine test is required – a difficult undertaking considering the diagnoses involved. According to literature, the urea cycle may be affected without producing variations in serum ammonia and diagnosis may be established based on urine orotate levels [34]. A hormone profile would be extremely welcome to aid in defining this complex metabolic context.

Another intriguing aspect revealed by this study is the insufficient respiratory compensation of established metabolic acidosis. Normally, drops in blood pH levels stimulate respiratory centers, causing respiratory acceleration to eliminate excessive CO_2 and lower ECO_2. In investigated patients, CO_2 levels appear normal. This may be justified by reporting concentrations to reduced fluid volume, as a result of hydric imbalance, or may be interpreted as a constant increase in plasma CO_2 caused by muscle activity in hyperkinetic or stereotypical patients (enforced by isolated increases of CK). On the other hand, a large number of patients (more than 40) receive antipsychotic, anxiolytic and anticonvulsive medication, which in turn reduce pulmonary ventilation (Garett and Grisham, 1999) [35]. Hypoventilation leads to respiratory acidosis, where carbon dioxide accumulates and produces carbonic acid, which subsequently dissociates into H^+ and HCO_3^-. Therefore, it may be concluded that, in patients receiving neuroleptic medication, acidosis is mixed, metabolically induced [36].

Among the side-effects of neuroleptic medication is liver toxicity, manifested in study patients by elevated liver enzymes, more significant in hepatitis B sufferers. Hepatitis B infection is only present in institutionalized patients and is likely underdiagnosed.

Conclusions

The study has confirmed an increased incidence of intellectual disability in male population, with a male to female ratio of 2,3:1.

Anemia frequently associated with intellectual development disabilities may be a cause of tissue hypoxia which in turn impedes cerebral function. In patients with available medical history, anemia was detected as early as postnatal periods, being associated with oxygen deprivation in perinatal stages. In such cases, persistent anemia requires permanent monitoring in order to compensate iron deficits through nutrition and treatment.

Metabolic acidosis is frequent in mentally retarded or ASD patients and is manifested by increases in anion gaps and osmolality, coupled with hydration deficits. If lactate levels rise above 5 mmol/L, metabolic acidosis may advance to lactic acidosis.

As the symptoms and pathology associated with development diseases are highly complex, diagnosis requires the corroborated activity of a multidisciplinary team that can also establish the best course of subsequent treatment. Keeping in mind the increasing occurrences throughout the world, in Romania ASD seem to be underdiagnosed, making the application of a unitary and early-detection algorithm most necessary.

Acknowledgements

Abbreviations

ASD = Autism Spectrum Disorders
CO = calculated osmolality
ECO_2 = total carbon dioxide (CO2) concentration
ESR = erythrocyte sedimentation rate
IQ = Intelligence Quotient
MR = Mental Retardation
Sat% = transferrin saturation (%)
TIBC = Total Iron Binding Capacity

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