Journal of
Clinical and Experimental Pharmacology

Melendez et al., Clin Exp Pharmacol 2013, 3:1
DOI: 10.4172/2161-1459.1000120

Research Article Open Access

Aluminium and Other Metals May Pose a Risk to Children with Autism Spectrum Disorder: Biochemical and Behavioural Impairments

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Abstract

Recent studies have shown that some toxic metals have been associated with neurological diseases. The binding and transport of these metals in the blood may occur by species of High Molecular Mass (HMM) and Low Molecular Weight Species (LMM) of proteins. These main species are known to be responsible for aluminum transporting is the case of transferrin and citrate. It seems that autistic children may have a genetic predisposition to accumulate large amounts of metals as it is has been proposed. This study seeks to understand the toxic mechanism of selected metals in autistic children and its correlation with the psycho-metabolic implications of the syndrome. Preliminary results have indicated that some metals such as chromium, arsenic and particularly aluminum were elevated in the blood of an autistic child, as compared to reference values of a normal child. A case-control study is under investigation. Furthermore, the consequences of the disease, as such the difficulties in socialization and language skill disabilities may also be related to the burden of toxic metals in general, particularly aluminum.

Keywords: Autism; Metals; Blood; Urine; Psycho-metabolic implications; Markers; Behavior

Introduction

The autism is a worldwide syndrome known as Autism Spectrum Disorders (ASD) or simply autism. As such, this neurological disorder is diagnosed in the early stages of childhood and also known as Pervasive Disorders of Development (PDD).

The incidence of autism in the 90's was estimated as 1 in every 110 children [1,2] but recent studies have been shown that this incidence is increasing to 1 in every 88 children [3] make it important to develop studies that give some insights on the possible causes and on the early diagnosis of this syndrome.

In addition to genetics, environmental factors seem to be related to this syndrome and the exposition to toxic metals may contribute for its development [3-6]. It seems autistic children have metabolic disorders that can be tiered accordingly. Therefore, it may affect the absorption nutrients processes and the excretion of toxic substances in the gastro-intestinal tract [7]. Thus, there appears to be no control on the nutrients uptake and probably a high amount of toxic metals is not excreted. Recent studies has investigated the relation of autism with toxic metals such as mercury, cadmium and arsenic [4,8]. Aluminum has not been blamed, as yet.

The availability of toxic metals may change trace element absorption and the interaction between essential elements and toxic metals can change threshold limits and pose a risk for toxic effects to cells [9]. Some elements such as cadmium, lead, mercury and aluminum may metabolically and nutritionally disturb the key role of essential metals (e.g., Cr, Zn, Mn, Fe, Mo, Cu) [10-13]. For instance, Fe deficiency increases absorption of cadmium, lead, and aluminum while lead interacts with calcium in the nervous system that can alter neurocognitive learning skills in the early stages of the child development. Furthermore, Cd and Al interact with calcium in the skeletal tissues to produce osteomalacia (bone pain, ribs, hips and vertebrae fractures) and muscle weakness whilst Pb can substitutes Zn on cell border enzymes and Cd has the strength to switch Zn in the cell metabolism. Calcium deficiency along with low content of Mg in the diet may play a role in aluminum-induced neurodegenerative diseases [14,15]. The role for highly aluminum-content in dietary food and water in the pathogenesis of encephalopathy is clinically characterized by dementia, asterixis, speech dysarthria/apraxia. Myoclonia and seizures are also well-known in dialysis patients although in Alzheimer`s disease remains a matter of discussion [16] while the effects of aluminum on the central nervous system were fully listed by Kawahara and Kato-Negishi [17] and shown in Table 1.

This may bring about consequences such as chronic inflammation of the digestive tract, changes in the immune system and in the brain [18]. As the most common consequences are the difficulty of socialization (prefers to be alone in an aloof manner) and language skill disabilities (unresponsive to normal teaching methods), as well as overweight [19].

The present work is being developed by a multidisciplinary project group that aims to integrate nutritional parameters with analytical chemistry, psycho-metabolic and clinical data. This paper has two strands, the chemistry analysis of blood serum and urine of an autistic child and the analysis of the protein profile of some metals in the blood as well as a psycho-metabolic study, for mapping the markers and to assist in the early diagnosis of infantile autism.

Materials and Methods

Subjects

This investigation has been approved by the National Ethical Research Committee Board under the no. 089/2010 with an additional amendment to the Protocol in 11/08/2011. In the first place, the parents were fully and duly explained about the aim of the research following an invitation to sign the Term of Consent and to fill a dedicated questionnaire adapted from Williams and Aiello [101] regarding the

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Received February 01, 2013; **Accepted** February 25, 2013; **Published** February 27, 2013

Citation: Melendez L, dos Santos D, Polido L, Mendes ML, Sella S, et al. (2013) Aluminium and Other Metals May Pose a Risk to Children with Autism Spectrum Disorder: Biochemical and Behavioural Impairments. Clin Exp Pharmacol 3: 120. doi:10.4172/2161-1459.1000120

	References
(1) Nucleus and gene expression.	
Binding to DNA	
Binds to histone-DNA complex and induces conformational changes of chromatin.	[20]
Induces topological changes of DNA.	[21,22]
Altered gene expression.	
Induces decreased expression of neurofilament and tubulin.	[23]
Induces altered expression of genes of neurofilament, APP, and neuron specific enolase.	[24]
Induces decreased expression of transferrin receptor.	[25]
Induces altered expression of RNA polymerase I.	[26]
Induces downregulation of mitochondrial cytochrome c oxidase.	[27]
Induces altered expression of calbindin-D28k.	[28]
Induces decrease in the expression of nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF).	[29]
Induces expression of pro-inflammatory genes and pro-apoptotic genes.	[30]
Induces elevated expression of APP.	[31,32]
Induces altered expression of oxidative stress marker genes (SOD1, glutathione reductase, etc.).	[33]
Induces decreased expression of neprilysin.	[34]
Induces altered expression of *β*-APP secretase (BACE1 and BACE2).	[35]
(2) Cellular functions	
Energy metabolismo	
Inhibits the activity of hexokinase	[36]
Inhibits the activity of phosphofructokinase	[37]
Inhibits the activity of glucose-6-phosphate dehydrogenase	[38]
Causes mitochondrial dysfunction and depletion of ATP	[39,40]
Decreases in activity and expression of TCA-cycle related enzymes (succinate dehydrogenase (SDH), alpha-ketoglutarate dehydrogenase (KGDH), isocitrate dehydrogenase-NAD$^+$ (IDH), fumarase (FUM), aconitase (ACN), and cytochrome c oxidase (Cyt C Ox)).	[41]
Phosphorylation and dephosphorylation	
Inhibits the activity of protein phosphatase.	[42]
Increases the activity of protein kinase C and cytoskeleton proteins.	[43]
Accelerates phosphorylation and accumulation of neurofilament.	[44]
Enhances Ca^{2+}/Calmodulin dependent protein kinase activity.	[45]
Accelerates phosphorylation of MAP 2 and neurofilament.	[46]
Inhibits dephosphorylation of tau.	[47]
Induces nonenzymatic phosphorylation of tau.	[48]
Abnormal accumulation of proteins	
Causes the conformational change and the accumulation of neurofilament and MAP1A, MAP1B.	[49]
Accelerates the phosphorylation of tau and its accumulation.	[50]
Causes the accumulation of tau protein in neuroblastoma cells or in primary cultured neurons.	[51,52]
Causes the accumulation of tau protein in experimental animals.	[53,54]
Causes neurofibrillary degeneration *in vivo*.	[55]
Causes the accumulation of A*β*P in cultured neurons or in neuroblastoma cells.	[56,57]
Causes the accumulation of A*β*P *in vivo*.	[58,59]
Neurotransmitter release	
Inhibits glutamate release.	[60]
Impairs synaptic transmission.	[61,62]
Inactivates glutamate dehydrogenase.	[63]
Inhibits NMDA-type glutamate receptor.	[64]
Inhibits choline acetyl transferase and tyrosine hydroxylase, glutamate decarboxylase.	[65,66]
Influences acetyl-CoA and inhibits acetylcholine release.	[67]
Activates monoamine oxidase.	[68,69]
Inhibits dopamine beta-hydroxylase.	[70]
Inhibits uptake of serotonin and noradrenalin in synaptosomes.	[71]
Channel inhibition	
Influences the activities of Na^+ channels and K^+ channels.	[72]

Enhances the voltage-activated Na^+ channels.	[73]
Inhibits the voltage-gated calcium channel.	[62,74]
Inhibits the IP^3-mediated Ca^{2+} release.	[75]
Others	
Influences GTP binding proteins as aluminum fluoride.	[76]
Inhibits GAP junction.	[77]
Inhibits axonal transports.	[78]
Binds to calmodulin and inhibition of calmodulin-binding enzymes.	[79]
Induces inflammatory responses.	[80]
(3) Membrane lipids	
Peroxidation	
Accelerates iron-induced membrane lipid peroxidation.	[81]
Enhances lipid peroxidation in liposomes.	[82]
Induces peroxidation of myelin lipids *in vivo*.	[83]
Increases peroxidation products (malondialdehyde).	[50]
Membrane properties	
Causes the change the lipid/phospholipids profiles of myelin *in vivo*.	[84]
Induces the change in membrane physical properties (surface potential, lipid fluidity, and lipid arrangement).	[83]
Induces the change of membrane fluidity.	[85]
(4) Higher functions	
Cell death	
Causes the apoptotic neuronal death.	[86,87]
Causes the apoptosis of astrocytes.	[88]
Causes the death of motor neuron.	[89,90]
Behavior, learning, and memory, others	
Inhibits long term potentiation (LTP).	[91,92]
Causes learning disorder or memory deficit in experimental animals.	[93–95]
Influences electrical activity in hippocampus and inhibits spatial learning memory deficit in aging rats.	[96]
Causes memory deficit in AD model mice.	[97,98]
Causes encephalopathy in dialysis patients.	[99]
Causes encephalopathy in patients with renal failure.	[100]

Table 1: Effects of aluminum on the development of the central nervous system.

daily habits and behavior of each child. All children who participate in this preliminary approach were diagnosed by a multi-professional team of physicians, psychologists, occupational and physical therapists and they had no signs or symptoms of chronic, degenerative nor infectious illnesses other than ASD. Amongst 38 children of both sexes at the age 3–12 years old, 12 (8 boys and 4 girls) met the criteria and were selected for this investigation. The inquiry enrolled questions of the mother's pregnancy and they were also followed-up since the early stages of development, along 6 years, uninterruptedly. The complete screen of clinical chemistry of these children was not displayed in this progress research report but they matched with normal reference values for non-autistic children. Only a child of this group had blood and urine analyzed while the other are under investigation.

The inclusion criteria were:

- No cerebral palsy dysfunctions
- No acute liver or kidney diseases
- No food or drink restrictions
- Under medication or not

The exclusion criteria occurred also when:

- Progressive neurological disorders
- Unstable epilepsy
- Physical handicap

Blood serum and urine analysis

The determination of the total concentration of metals in blood serum and urine of an autistic child was performed by Inductively Coupled Mass Plasma Spectrometry (ICP-MS). The concentration metals determined were Mg, Al, Cr, Mn, Ni, Cu, Zn, As, Se, Mo, Cd, Sb, Pb and U. The samples were diluted in nitric acid 2% before analysis.

Before analyzing the sample, the methodology has to be validated.

For this, recovery tests were performed using certified material of blood serum and recovery approximately of 90% was found.

It was performed a qualitative study in order to evaluate the capability of elements to bind proteins in biological fluid samples, using size exclusion chromatography. Was used a Superdex peptide column, and was possible to separate de species of blood sample in two fractions: High molecular mass (HMM) and Low molecular mass (LMM). The HMM fraction contain albumin, transferrin and immunoglobulin G. The fraction of LMM basically consists of citrate and phosphate. The monitoring of proteins was performed by UV (ultraviolet) and the metals analysis was made with ICP-MS [102-105]. Recovery tests were performed with the column in order to verify if it would not be contaminating the samples or retaining Al. For this tests, a mixture of standards (transferrin, albumin and immunoglobulin G), simulating HMM in blood was used. In an aliquot it was added 6 μg L^{-1} of Al.

This study was performed only for some metals. The essential (Cu and Cr) and toxic (Al and Pb) elements were chosen.

Psycho-metabolic markers

The methodology for the psycho-metabolic study was based on the application and interpretation of questions to parents of the selected autistic children. There were several questions about pregnancy and childbirth, and also about the child's cognitive, psychomotor, language and emotional developments in different ages and stages of the child. There were different questions for each development phase.

This instrument was elaborated from the Inventory and of the scale adapted from Williams and Aiello [101], which was carried out throughout the study and with a brief analysis about the familiar characteristics, dwelling aspects of house-keeping, the school, the caretaking, which involves the relative and mediating aspects that can relate the school performance of the child.

The questionnaire developed for the analysis and establishment of psycho-metabolic markers takes into consideration the assessment to personal data of the parents that includes: economic aspects, characteristic of the domestic and household environment, relative aspects to the feeding, pregnancy besides some factors that say respect to the some characteristics of the routine and the behavior of a child. In such a way, the instrument for analysis of the psycho-metabolic markers does not take into consideration the caretakers and/or educators, although it has taken a glance to appreciate the pertaining to school performance and, therefore, its attention is in the nutritional and manner aspects of the family, specially the mother and the child, since it enrolled subjects from birth up to twelve years of age.

Results and Discussion

Blood serum and urine analysis

The results from essential and toxic elements concentration of urine and blood serum of an autistic child compared to reference values are shown in Table 2. Even considering that these results refer to samples of only one child, some preliminary important aspects must be pointed out. In the blood serum samples the essential elements concentration such as Zn, Cu and Mg all are below the threshold. On the other hand, Al, Cr and As concentration, considered as toxic elements, showed much higher values than reference ones. This result corroborates with the hypothesis of Mulloy et al. [7], which raising the possibility that accumulation of toxic metals and eliminate micronutrient. The association of the low concentration of Mg and high of aluminum corroborates results from literature [14,15] that relates the low concentration of Mg with aluminum-induced neurodegenerative diseases. Regarding to urine sample, Al together with Zn, also showed higher concentration compared to reference ones.

Figure 1 shows the results of metals association with proteins after fractionation with a size exclusion column in the blood serum sample. The first peak (Figure 1a) at about 11 min refers to the fraction of High Molecular Mass (HMM), which contains albumin, transferrin and immunoglobulin G. The second peak at about 32.5 minutes (Figure 1a), refers to the fraction of Low Molecular Mass (LMM). The results show that all the metals monitored bind with the fraction of HMM (Figure 1b), highlighting the fact that only aluminum was also associated to the fraction of LMM, indicating that this metal has besides the ability to bind to proteins contained in HMM, also a different transport mechanism in the blood comparing to the others metals studied. Studies are being performed in order to identify which specific protein is binding to the metals in each fraction.

	Blood serum		Urine	
	This study	**Reference value**	**This study**	**Reference value**
	Concentration (mg /L)			
Zn	1.130 ± 0.0 15	35.4-88.7	2.250 ± 0.006	0.266-0.846
Cu	0.7473 ± 0.011	0.80-1.60	0.0288 ± 0.004	0.0042-0.050
Mg	0.8700 ± 0.016	16.00-255.0	8.150 ± 0.005	nf
Se	0.2301 ± 0.004	0.580-2.340	0.0991 ± 0.006	0.001-0.20
	Concentration (μg /L)			
Al	14.95 ± 0.20	0.3-7.5	122 ± 1.57	1-3
As	42.32 ± 1.43	0.2-6.2	38.62 ± 3.66	10-50
Cr	30.09 ± 1.01	0.02-0.35	17.9 ± 2.46	nf
Pb	nd	1.5-3.0	1.18 ± 0.003	nf
Ni	nd	1.0-28.0	34.42 ± 1.34	nf
Cd	nd	0.1-1.10	0.12 ± 0.02	nf
Mo	nd	nf	127.9 ± 7.63	nf
Mn	nd	1.5-22.0	nd	nf
Sb	nd	nf	nd	nf
U	nd	nf	nd	nf

nf=not found nd=Not detected

Table 2: Elements concentration (μg L^{-1}) and standard deviation in blood serum and urine of an autistic child compared to reference values [106,107].

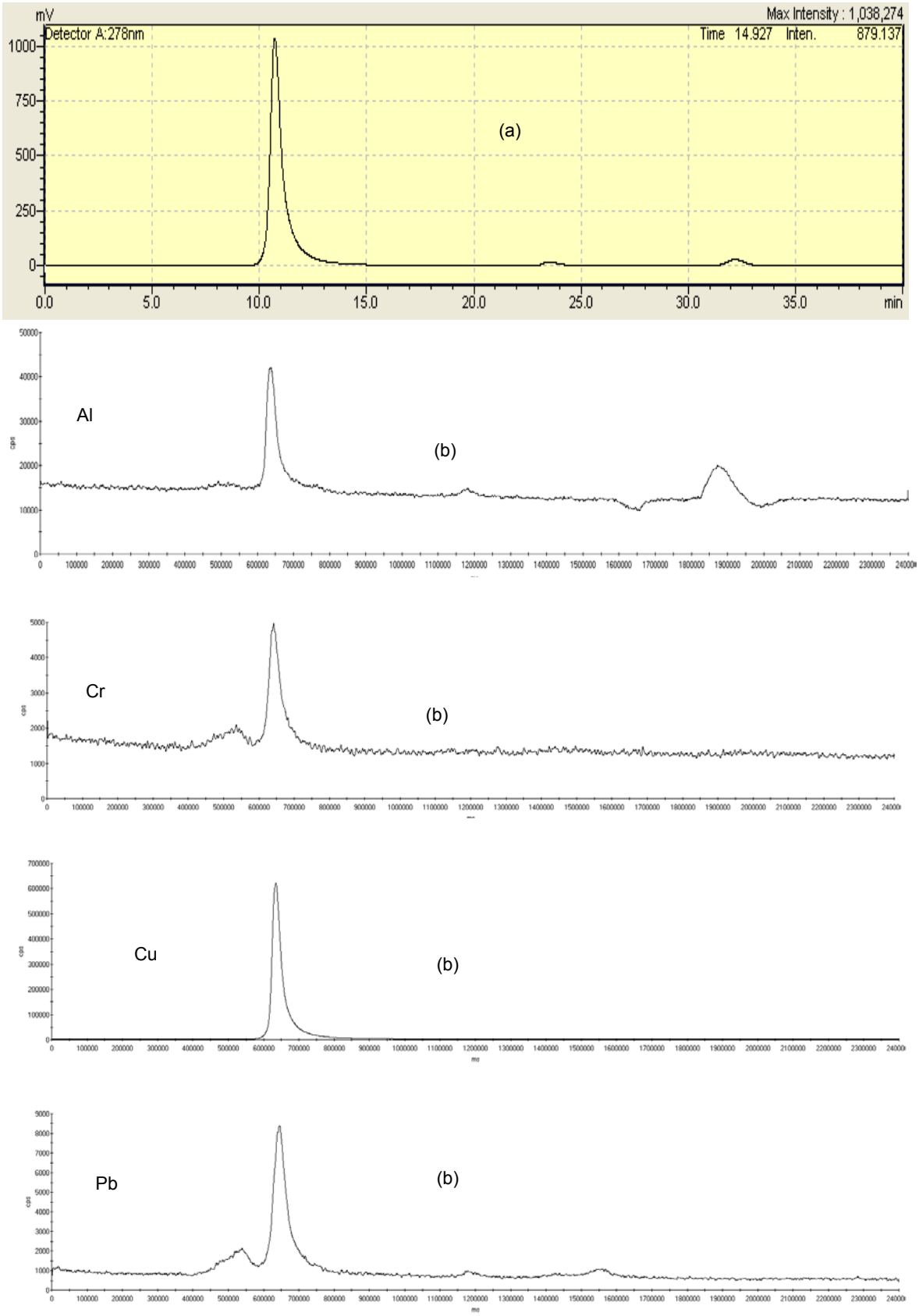

Figure 1: Blood serum analysis of the autistic child with SEC. (a) Fractions of HMM and LMM obtained by UV. (b) Metals associated with each fraction, obtained by ICP-MS.

Psycho-metabolic markers

The changes and adaptations have been made in the questionnaires in order to carry through a supportive tool that aimed the inclusion of every necessary aspect in this inquiry without disregarding the possibility of the mother and/or the child is contaminated by the ingestion of food or drink or even by a single contact with noxious substances. These may be the triggering factors that contribute for metabolic alterations and disturbances in the untainted child to uncurl the infantile phases of development.

Amongst the samples already analyzed and the studied aspects, it can be that for the pregnancy and childbirth study (Figure 2), the results show that 70% of mothers took vaccine or injection and 80% of them ate canned food and fish during pregnancy. About babies at birth 70% were considered quiet when placed in the cradle, 80% of them was easy to care and 90% received all vaccines. In this study was possible to identify two important data regarding exposure to toxic metals. In 80% of cases the autistic children have used or make use of controlled drug and 90% of them have taken all vaccines.

The results for the developmental stages (Figure 3), show that from zero to six months is already possible to observe early signs of autism. For this phase, two questionnaire items should be highlighted: 50% of the children cry a lot and 60% of them present absence of motion hold. From six to twelve months the marker related to language development indicates that 80% of children have difficulty to speak a simple word. From 1 to 2 years, cognitive and psychomotor markers already show an increase, with the emphasis on the fact that 90% of children do not chain two actions. From age 3 all the markers already present results greater than 50%. Two issues are highlighted in this step: obsession with some object and rotate objects in a peculiar way, both with 90%. At age 4, the cognitive and psychomotor markers reach their maximum values. Is when the disease is more easily detected. At this age 100% of children are considered either extremely agitated or passive, and 90% of them show repetitive behaviors. At 5, the highlight involves the cognitive and the affective markers. At this age, 83% of autistic children challenge obedience. From six years old to teenage years in one hundred percent of the cases, the language is limited.

The diagnosis of Autism Spectrum Disorders (ASD) is nowadays almost exclusively based on clinical outcomes, mostly concerning aspects that often takes into consideration the social communication skills, repetitive behaviors and restricted area of interest of the child. However, diagnostic tools that may reveal each of these changes in cognitive and social behavior have to bridge the gap for starting the proper treatment of these children. From birth to the early stages of childhood, parents should be aware of the prime signs and symptoms of their autistic infant and these needs highlights the importance in applying an assertive parent's inquiry and a full psycho-metabolic approach as precociously as possible. Thus, these will allow caretakers to develop or exercise the social and learning abilities of the child as well as the functional speech skills besides knowing the bioavailability of metabolic markers. An early diagnosis makes possible the occurrence of also precocious therapeutic treatment which corroborates for a more satisfactory prognostic of this individual.

Preliminary Conclusions

The understanding on how metals are associated to proteins and their blood transport mechanism in autistic child is essential to biomedical area, since these data may contribute for new treatment discovery.

In this study was possible to notice that the concentration of metals in urine and mainly in blood, of autistic child were outside the range, indicating a possible correlation between these found changes and the disease.

The results from the study of proteins bind metals shows that Al has a peculiar behavior in the serum blood of an autistic child, been the only one that appears bind to LMM fraction (39% of the Al total), whereas for the others metals, Cu, Cr and Pb not.

Regarding to psycho-metabolic study it is possible to point out the importance of precocious markers determination. The difference between small signals and symptoms already established can be subtle in the first months of life, but its development without no assistance or knowledge can develop manifestations, bigger commitments and deficits each time. The analysis of the partial results also allows an inquiry that it exceeds the child characteristics that, it is possible to make an appreciation on the profile of the mothers. The results showed that the mothers had contact with toxic substances or agents, either through the ingestion of medication, vaccines or of the anesthesia used during childbirth. These data may indicate a relation between the maternal degree of toxicity and the development of signals of the autistic spectrum.

A case-control study is underway in order to further investigate that aluminum may be related to an event that probably triggers the genetic predisposition to the typical behavior that is diagnosed

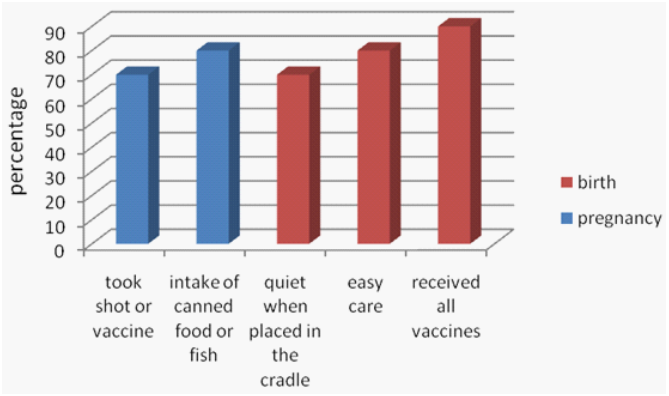

Figure 2: Psycho-metabolic study: Pregnancy and childbirth.

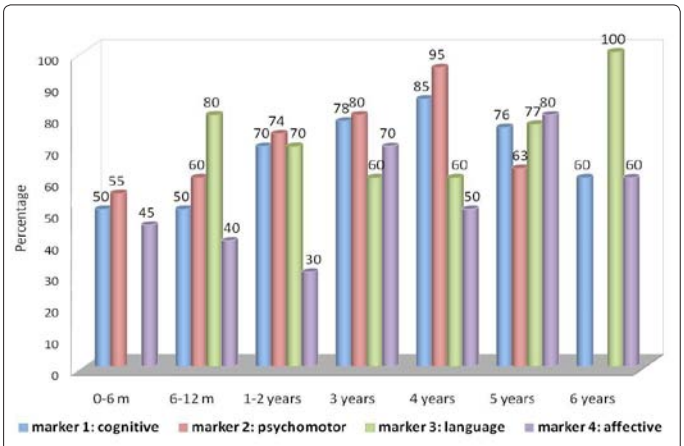

Figure 3: Psycho-metabolic markers versus developmental stages.

as autism, in the very early stages of life while the child is still in the womb. In other words, and tragically, the mother is the immediate sources of the contamination. Once these preliminary evidences have more forthrightly established the link between aluminum and the occurrence of autism, one might take into consideration a study where mothers are the cases and controls. Other toxic metals such as arsenic and in particular mercury may pose a great relative risk of autism. The air contamination of mothers that gave birth to autistic children in geographic areas that had a higher levels of ambient mercury have been associated to the high level of emissions of this metal from sources such as coal-fired power and cement (kilns) plants [108].

The project is not finished, but nevertheless more results are necessary to confirm the interference of metals in the children's cognitive and psychomotor development, but the analysis of the psycho-metabolic markers, might suggest a relation between the level of toxic metals and the development process of the autistic children.

Acknowledgement

The authors would like to thank INCT-TMC Ocean 573-601/2008-9 by financial support and *Tocando em você* school.

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