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The Relationship of Aluminium and Silver to Neural Tube Defects; a Case Control

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Abstract

Objective: The purpose of this study was to identify the relationship of neurotoxic inorganic elements in the hair of patients with the diagnosis of Neural Tube Defects. Our initial hypothesis was that neurotoxic inorganic elements were associated with Neural Tube Defects.

Methods: Twenty-three samples of hair from newborns were obtained from the General Hospital, "Aurelio Valdivieso" in the city of Oaxaca, Mexico. The study group included 8 newborn infants with neural tube pathology. The control group was composed of 15 newborns without this pathology. The presence of inorganic elements in the hair samples was determined by inductively-coupled plasma spectroscopy (spectroscopic emission of the plasma).

Findings: The population of newborns with Neural Tube Defects showed significantly higher values of the following elements than the control group: Aluminium, Neural Tube Defects 152.77±51.06 µg/g, control group 76.24±27.89 µg/g; Silver, Neural Tube Defects 1.45±0.76, control group 0.25±0.53 µg/g; Potassium, Neural Tube Defects 553.87±77.91 µg/g, control group 341.13±205.90 µg/g. Association was found at 75 percentile between aluminium plus silver, aluminium plus potassium, silver plus potassium, and potassium plus sodium.

Conclusion: In the hair of newborns with Neural Tube Defects, the following metals were increased: aluminium, silver. Given the neurotoxicity of the same, and association of Neural Tube Defects with aluminum and silver, one may infer that they may be participating as factors in the development of Neural Tube Defects.

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Key Words: Aluminium; Silver; Hair; Newborns; Neural Tube Defects

Introduction

Among congenital malformations, Neural Tube Defect (NTD) cases occupy a special place because of the implicit damage that occurs in the different structures of the nervous system, damages which render them incompatible with life. The most common types of NTD are anencephaly, spina bifida and encephalocele[1], all of which represent 95% of the cases[2]. In spite of the fact that in many

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subjects, the implied mechanisms are little known with precision and certainty, the diverse genetic, molecular and epidemiological studies have been able to relate the NTD with environmental teratogens such as drugs, chemical compounds, infectious agents, the ingestion of caffeine and alcohol, exposure to X rays during pregnancy, genetic factors, mutations, chromosomal abnormalities, and a deficit in maternal ingestion of folic acid. Among environmental pollutants that have been suggested as related to NTD are vinyl chloride, solvents, agricultural chemicals, water nitrates, and metals, among others.

In the last decade, much attention has been directed toward heavy metals, and especially, neurotoxic metals[3]. In the case of pregnant women and the newborn, there have been few studies about the levels of presence of heavy metals (including cadmium, lead, zinc, selenium, copper, mercury, manganese, and nickel) in these populations[4-8].

The inorganic elements that are important from the neuorotoxic point of view are: arsenic (As), lead (Pb), thallium (Tl), mercury (Hg), aluminium (Al), chromium (Cr), manganese (Mn), and nickel (Ni); other inorganic elements that are relevant from exposure during organogenesis, that may cause fetal anomalies are: boron (Bo), cadmium (Cd), cobalt (Co), copper (Cu), gallium (Ga), lithium (Li), silver (Ag), selenium (Se), uranium (U), vanadium (V), and zinc (Zn)[9-11].

Aluminium is the metal most widely distributed in the environment, and is widely used in daily life. Exposure to this toxic metal occurs through air, water and food. It has been reported that aluminum has toxic effects on humans. Some cases of aluminosis (in addition to pneumonia or chronic bronchitis) have been manifested in illnesses of the central nervous system such as ataxia and dementia[12]. Aluminium is involved in the etiology of several neurodegenerative diseases. For example, aluminium adversely affects the spatial learning and memory abilities, and it reduces nerve synapses[13]. Aluminium is also related to oxidative stress-related damage to lipids, and membrane associated proteins[14]; aluminium is likewise related to changes in the intracellular calcium messenger system[15]. Moreover, aluminum inhibits the Na^+/K^+ ATPase activity in brain[16]. Failure of the Na^+/K^+ ATPase has been implicated in the pathophysiology of neurodegenerative diseases.

The environmental contamination with silver allows this element to be absorbed into the body through the lungs, the gastrointestinal tract, the mucus membranes of the urinogenital tract, and through the skin[17]; hence, silver is found in myocardium, mucous membrane, kidney, liver, and many areas of the brain[18], and produces deficits in learning and memory. Silver also binds itself to high-molecular-weight proteins and the metallothionein fractions[19]. The silver penetrates the blood-brain barrier and accumulates into large neurons in the brain stem and spinal cord[20]. Silver also induces a decrease in the total volume of hippocampal pyramidal cells[21]. Silver nanoparticles may interact with the cerebral microvasculature, producing a pro-inflammatory cascade and then induce brain inflammation and neurotoxicity[22]. Also silver at low nanomolar concentrations strongly inhibits the activity of large-conductance Ca^{2+} activated K^+ channels[23].

Environmental exposure to toxins, by seminal fluids and the contamination of work clothes worn home, can cause a secondary exposure in parents, and produce genetic damage by gene-environment interaction, or a mutation of germinal cells in which mutagenesis may be expressed in subsequent generations; this may happen before or after conception by means of direct action on the embryo or by embryo placenta complex, teratogenesis. Although exposure prior to conception is important, the greatest risk of teratogenesis via exposure of the mother is related, generally, to exposure during the phase of organogenesis.

The aim was to identify neurotoxic heavy metals, as those mentioned above. In this study, a small group of newborns with neural tube defects, with parents who have confirmed exposure to environmental contaminants, were studied, and compared with newborns without neural tube defects.

Subjects and Methods

Approval to conduct the studies was provided by the Ethics Committee from the Hospital "Aurelio Valdivieso" in the city of Oaxaca, Mexico.

Twenty three (8 females, 15 males) recently born, between the ages of 24-48 hours were studied. The group was comprised of 8 newborn cases (6 females and 2 males) with neural tube defects (case); the control group was comprised of 15 newborns (5 females and 10 males) without pathology, all originating from different communities of the state of Oaxaca. All the newborn with neural tube defects had parents who had confirmed exposure to environmental contaminants. All the newborns included in the study had received folic acid during the pregnancy.

Design of the Study

The study was prospective and was approved by the Planning and Regional Development doctoral committee of the Technological Institute of Oaxaca, Mexico. A written letter of consent was obtained from the parents. Parents were given a questionnaire in order to compile data which included: age and weight of the mother; occupations of both parents, consumption of abuse substances; type of water consumed; the type of utensils used in cooking; and the type of fuel used.

Approximately 2 cm of distal hair from the occipital region of the head were cut with titanium scissors. The hair specimens (previously labeled) were collected in plastic bags with a hermetic closure, previously labeled, and stored at room temperature, 1.0 g of the hair obtained was submitted to a first washing with the detergent Bio-Klean, (Saf-Care, China), and then the samples were rinsed 3 times with distilled water and one with acetone at 10% (analytical grade, Merck, Darmstadt, Germany). Finally, the hair was rinsed repeatedly with distilled sterile water and the samples were dried in a Thermolab (Thermolab, S.A. de C.V. Mexico) oven at 60°C. Then 0.5 g of finely reduced hair was weighed on a scale (Analytical Balance, Single-Pan, Substitution-Mettler H10, with a range of 0.1 mg, Mettler Instrument Corporation Princeton NJ. USA). The hair samples were then incinerated in a furnace muffle (West Instrument Corporation, USA, DGE-5825 NOM-1, with a range of 0 a 1400°C), at 600°C until the calcination was completed, around 2 hours. The ashes were suspended with 10 ml of HNO_3 65% p.a., (Merck, Darmstadt, Germany), and then heated 10 minutes; afterwards, 10 ml of distilled water was added, and the samples were re-heated for 10 more minutes, and then were transferred to a volumetric flask of 50 ml for measurement and filtering. All the materials used were previously washed with double-distilled and de-ionized water.

For the analytical determinations of the heavy metals a spectrophotometer of emission analysis of plasma, coupled with inductively ICP-AES, Thermo Elemental, model Iris Intrepid 460 (Franklin, USA), was used, to which a system of generation of plasma of the gas Argon compressed to a pressure of 80 psias with a purity of 99.995% was coupled. For the calibration curves, auto samplers at a temperature of -46°C employing accused patterns in 1.5% nitric acid, were used. The curve equation of the work was: Ap=0,010 c (correlation coefficient, r=0,9998); where Ap is the absorbance peak height and c the quantity of metal in ng. Each sample was analyzed in duplicate, obtaining the average, the standard deviation, and the relative standard deviation.

The aqueous standards of the calibration curve were prepared beginning with an internal multi-element standard AccuStandar Inc. (New Haven USA). All the solutions were prepared with double-distilled and de-ionized water, grade I ASTM (American Society for Testing Materials & Methods), with an electric conductivity >16,6 MW cm^2 to 25ºC. The standards were prepared diluting the multi-element reagent with certified water, high-purity, with a concentration of µg/ml in HNO_3 to 5%, 65% P.A., (Merck, Darmstadt, Germany). The range of concentration was 0.005-0.5 (µg/ml)[24].

The statistical analysis was achieved with a confidence level of 95%, with a level of significance of α=0.05. Logarithmic transformations were used to normalize data of abnormal distribution; the Mann Whitney test was used to compare the groups. To identify the interaction of inorganic elements, all the cases selected had an increase over the 75 percentile; Fisher´s exact test was used to identify interaction. All analyses were performed using GraphPad

Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com.

Findings

Among the parents of children with NTD, the arithmetic mean age for mothers was 20 years (with a standard deviation of 2.2), while that of fathers was 32 years (with a standard deviation of 2.5); the mean weight of mothers was 65.5 kg (with a standard deviation of 6.1), and that of fathers 67.3 kg (with a standard deviation of 9.6). In substance abuse, tobacco consumption was found in only one of the parents; the mothers' occupation was housewife; fathers' occupations varied from: brick layer (1 in 8); painter (1 in 8); mechanic (1 in 8); sexton (1 in 8); intendant (1 in 8); and diverse occupations (3 in 8).

All had had some connection with some sort of environmental contaminant. All families live in a place near a minerals region zone. The source of water for daily use was potable water in all the cases. Among cooking utensils, pewter measured 4 in 8; the use of aluminium utensils was 3 in 8. All families used gas LP (propane/ butane) as fuel. The detected elements that showed significantly higher values in newborns with NTD (in contrast to healthy newborns) were aluminium, silver and potassium (Table 1). Among the 31 elements that constituted analysis by ICP, we did not find in the population with NTD significant increases of arsenic, barium, beryllium, cadmium, cobalt, copper, chromium, tin, iron, manganese, mercury, molybdenum, nickel, lead, selenium, thallium, vanadium, and zinc.

In four cases we found that inorganic elements were increased over the 75 percentile; the combinations were aluminium plus silver, aluminium plus potassium, silver plus potassium, and potassium plus sodium (Table 2).

Discussion

Given that currently all pregnant women are treated with folic acid, other factors that may be related to NTD may be considered, especially environmental factors. The increase in aluminium, and silver in the hair of newborns with NTD lead us to consider first of all environmental contamination. As in many other countries, in the state of Oaxaca, Mexico, there is a great diversity of minerals. In 14 mineral-region zones in the State of Oaxaca, there are 6 that are currently in

Table 1: Inorganic elements in hair samples of healthy newborns and those with neural tube defects (NTD)

Inorganic elements	Case (n= 8) Mean$_G$ (SD) µg/g	Controls (n= 15) Mean$_G$ (SD) µg/g	*P* Value*
Aluminium (Al)	152.77 (51.06)	76.24 (27.89)	0.005
Barium (Ba)	59.57 (55.07)	47.02 (55.05)	0.5
Calcium (Ca)	3259.87 (961.67)	3186.00 (992.25)	0.9
Copper (Cu)	109.96 (53.41)	165.14 (320.42)	0.5
Strontium (Sr)	6.03 (2.24)	7.61 (2.31)	0.1
Iron (Fe)	75.52 (31.39)	81.80 (31.54)	0.5
Phosphorus (P)	379.27 (97.04)	302.76 (112.64)	0.1
Magnesium(Mg)	295.91 (125.93)	248.20 (113.69)	0.2
Manganese (Mn)	2.08 (1.51)	5.44 (12.55)	0.5
Nickel (Ni)	4.13 (2.45)	4.08 (2.04)	1
Silver (Ag)	1.45 (0.76)	0.25 (0.53)	0.003
Lead (Pb)	4.53 (2.32)	4.8 (2.27)	0.8
Potassium (K)	553.87 (77.91)	341.13 (205.90)	0.01
Sodium (Na)	5821.25 (2446.3)	4905.66 (1780.8)	0.4
Titanium (Ti)	1.65 (0.97)	0.91 (1.57)	0.1
Zinc (Zn)	205.26 (53.17)	194.65 (48.89)	0.4

G: Geometric Mean, Mann Whitney test / * *P* value<0.05 for the case–control comparison was significant.

Table 2: Interaction of inorganic elements in the cases of neural tube defects

	Odds ratio	*P* Value*
Aluminium + silver	42.43	0.003
Aluminium + potassium	17.18	0.04
Silver + potassium	17.18	0.04
Potassium + sodium	17.18	0.04

‡ Fisher exact test, 95% confidence interval, two tailed

operation[25], that could be associated with environmental pollution.

In this work we proposed to identify if the presence of inorganic elements that have been reported as neurotoxic, is elevated in the hair of newborns with NTD.

The increase in potassium in the group with NTD suggests a possible alteration in the physiology of the same.

Unlike previous studies done in humans[4-8], we have found two metals that are associated with neural tube defects.

Aluminium toxicity is associated with NTD of rat embryos. In neurodegenerative diseases such as Parkinson's, dementia, and motor neuron disease have been reported aluminium hair in concentrations similar to the population[26]. The aluminium is a dysmorphogenesis agent, which inhibits embryonic development[27]. The increase in aluminum among the newborns with NTD could be associated with the pathology of the nervous system.

Moreover, medical literature has not described the association between neural tube defects and the presence of high levels of silver. There are several reports that described a weak relationship to women who used mercury occupationally as dental assistants and spontaneous abortion, with reduced fertility and neural tube defects. These works seek to point out the participation of mercury, regardless of its content as silver-mercury[28].

Moreover, given the similar size between Ag+ and cations such as Na+ and K+[17], it is possible that the presence of silver interacts and competes with ion channels[29], which explains the increase of potassium in this study.

It is interesting to note the association of elements found in the 75 percentile in cases of neural tube defects that are not seen in any subject in the control group. These environmental associations, however, are insufficient to explain the association between silver plus potassium, and potassium plus sodium.

A limitation of this study is the small sample size. Given the pervasiveness of these problems in familiar, social, and economic ambits, it is necessary to continue investigating, in a larger population, the association of inorganic elements in the development of these pathologies. It is also necessary to inform the medical community and areas of environmental pollution about these studies, in order to prevent neurotoxic agents in pregnant women.

Conclusion

Our results suggest that the presence of metals of higher values that were detected in the study group, in contrast to the control group, could be risk factors in neural tube pathologies.

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Conflict of Interest: None

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