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Heavy Metals and Trace Elements in Blood, Hair and Urine of Nigerian Children with Autistic Spectrum Disorder

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ABSTRACT

Autism spectrum disorders (ASD) are a group of developmental disorders defined by a range of behavioral problems including social withdrawal, communication deficits, and stereotypic/ repetitive behavior. Patient observation and clinical history rather than biomarkers as known in laboratory analysis are the defining factors. Pathophysiological etiologies remain controversial, but genetic and environmental factors have been discussed in recent years. International research has focused on neurotoxic metals such as mercury and lead, suggesting that these and other metals contribute to the development of the disorder.

Since the Niger-Delta region of Nigeria is widely known for its petroleum industry and pollution, we aimed to evaluate if Nigerian children diagnosed with ASD carry a greater burden of toxic metals compared to healthy Nigerian children living in the same region. While the ASD group shows a higher metal concentration in blood and hair, combined with low blood zinc levels, we also determined an unusual metal burden in the healthy group but no zinc deficiency.

Keywords: Autism, Toxic Metal Burden, Zinc, Niger Delta.

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Introduction

Autism is a severe developmental disorder which involves social withdrawal, communication deficits, and stereotypic/repetitive behavior. The neurological etiologies which precipitate autism symptoms remain controversial, but both genetic and environmental factors (and their interactions) have been implicated. One environmental factor that has received significant attention is the body burden of mercury, lead, and other toxic metals (Bernard 2001; DeSoto 2007; Bradstreet2003). Environmental factors such as petroleum pollution and heavy metals were implicated in the development of ASD by Volk *et al*, Roberts and other researchers. Our previous studies on Arab and Indian children confirmed this (Blaurock-Busch 2011).

The Niger - Delta Region of Nigeria is a petroleum-rich region with a history of petroleum exploration, gas flaring and oil pollution where toxic waste dumping has been a problem for decades.. Oil spills are common and Nigerian regulations of the oil industry are weak and rarely enforced. (Baird 2010). Half of all spills occur due to pipeline and tanker accidents, other causes include sabotage (28%) and oil production operations (21%), with 1% of the spills being accounted for by inadequate or non-functional production equipment. (Nwilo 2001) Such industrial practices contribute to heavy metal exposure as has been documented by studies in the Arab Gulf Region (Freije 2015).

Exposure to environmental risk factors may affect tissue concentration of heavy metals and trace elements, and thus potentially contributes to the genesis of ASD. Hence, we aimed to examine the metal burden of Nigerian ASD patients versus a healthy control group, all living in the same vicinity. We used hair analysis, which is a diagnostic tool for the detection of long-term exposure, (Al-Ayadhi 2005) and tested blood and urine for the detection of immediate metal exposure, (Blaurock-Busch 2010). The main purpose of this study was to examine present and past environmental risk factors that may relate to problems in Nigerian children with autism spectrum disorder. We also tested a healthy control group living under similar conditions.

METHODS:

Laboratory diagnostics allow the distinction between present and past metal exposure. If an acute exposure as diagnosed in blood remains for weeks or even longer periods, and if the renal metal elimination is inadequate, it is assumed that the increased intake and decreased output contributes to tissue accumulation as reflected in hair.

We assessed the levels of trace elements and heavy metals in hair, blood and urine of the autistic and control group, aiming to establish a link between environmental exposure and the genesis of autistic spectrum disorder. We reasoned that a comparison of blood, urine and hair values would confirm that both present and past exposures are a potential cause of a patient's metal burden. In addition, we evaluated if zinc deficiency is a contributing factor.

For this study, we selected people from the Niger Delta Region, a densely populated area that covers 20,000km², and is home to a population of 20 million.

Samples collection and analysis

Blood, urine, and hair samples were collected from participants and shipped overnight to Micro Trace Minerals Laboratory in Germany. Due to shipping problems, not all samples arrived intact. Samples not suitable for testing were discarded. All samples were analyzed with inductively coupled Plasma Mass Spectrometry (ICP-MS), using the Agilent ICP-MS 7500 with Octopole Reaction System (ORS). Table 1 shows the elements tested and respective isotopes.

For this study, we recruited a total of sixty-seven participants, all from the Niger Delta Region of Nigeria. The ASD group initially

consisted of 48 patients who had been professionally diagnosed with Autism Spectrum Disorder. Their age ranged from 3 to 25 years of age, the mean age was 9.7years. The Control Group consisted of 19 healthy participants, and their age ranged from 4 to 19years of age with a mean age of 9.7 years.

A total of 46 urine samples were accepted for testing. Of these, 39 samples were from the autistic group, 9 females and 30 males. From the control group, 7 samples were tested, 4 males and 3 females.

Thirty-seven blood samples (8 females and 29 males) were accepted from the autistic group. For the control group, 7 samples (3 females and 4 males) were tested.

Also 61 hair samples were collected, 43 hair samples came from the ASD group (12 females and 31 males) and 18 samples from the control group (10 females and 8 males).

This study was approved by the Health Research Ethics Committee of the Federal Ministry of Health, Abuja, Nigeria (Approval Ref. No. NHREC/01/01/2007) and followed guidelines of the Helsinki Declaration.

BLOOD

Blood samples (5 ml) were drawn by a qualified medical practitioner from an arm vein (after a twelve-hour overnight fast) into EDTA tubes while keeping the subject in a sitting position throughout the procedure. Of that 1ml of EDTA blood was acid digested with non-ionic nitric acid and diluted to 5ml with deionized Millipore water. These samples were analyzed for metal content using inductively coupled Plasma Mass Spectrometry (ICP-MS).

URINE

Spot urine was collected in clean plastic tubes and 1ml of urine was acid digested with non-ionic nitric acid, diluted to 5ml with deionized Millipore water and analyzed for metal content using inductively coupled Plasma Mass Spectrometry (ICP-MS).

The metal concentration was reported in mg/g creatinine for the macro elements and mcg/g creatinine for trace elements, including toxic metals. Normalization per mg or mcg creatinine reduces the potentially great margin of error which otherwise can result from sample collection and variation in sample volume.

HAIR

Hair samples cut 3-5cm from the scalp were washed with non-ionic detergents and rinsed with deionized Millipore water before drying in a special, designated oven. The washed and dried hair was weighed close to 100mg, before it was acid digested with non-ionic nitric acid, diluted to 5ml with deionized Millipore water and analyzed for metal content using inductively coupled Plasma Mass Spectrometry (ICP-MS).

STATISTICAL ANALYSIS

All statistical analyses were carried out using SPSS (version 22). Descriptive statistics were used to determine the mean, standard error of mean for each of the parameters tested.

RESULTS

In this paper, attention is given to the potentially toxic elements; aluminum, barium, mercury, nickel, and lead, because an unusual percentage of pathological results was noted for these elements in both groups. We also evaluated the manganese and zinc status of both groups.

The concentration of blood and urine elements was compared to reference ranges (RR) as given by the German environmental agency (Umweltbundesamt). For RR not given, laboratory ranges were used as developed by Micro Trace Minerals laboratory. This also pertains to hair RR. All RR are based on a 95 Percentile of a so-called healthy population. All laboratory rules and regulations were followed.

We acknowledge that the limited number of controls does not allow for a sufficient comparison of both groups. However, our data demonstrates that for the metals aluminum, barium, manganese, mercury, nickel and lead an unusual percentage exceeded RR as outlined in Table 2 to 4.

Table 1: Isotope listing of elements tested

Element	Symbol	Isotope	Element	Symbol	Isotope
Lithium	Li	7	Tin	Sn	118
Beryllium	Be	9	Antimony	Sb	123
Boron	B	10	Iodine	J	127
Magnesium	Mg	24	Cesium	Cs	133
Aluminum	Al	27	Barium	Ba	138
Calcium	Ca	44	Lanthanum	La	139
Titanium	Ti	49	Cerium	Ce	140
Vanadium	V	51	Praseodymium	Pr	141
Chromium	Cr	52	Neodymium	Nd	146
Manganese	Mn	55	Samarium	Sm	147
Iron	Fe	56	Europium	Eu	153
Cobalt	Co	59	Gadolinium	Gd	157
Nickel	Ni	60	Dysprosium	Dy	163
Copper	Cu	63	Erbium	Er	166
Zinc	Zn	66	Thulium	Tm	169
Gallium	Ga	69	Ytterbium	Yb	172
Germanium	Ge	74	Lutetium	Lu	175
Arsenic	As	75	Hafnium	Hf	178
Selenium	Se	78	Tantalum	Ta	181
Rubidium	Rb	85	Tungsten	W	182
Strontium	Sr	88	Rhenium	Re	185
Zirconium	Zr	90	Iridium	Ir	193
Niobium	Nb	93	Platinum	Pt	195
Molybdenum	Mo	98	Mercury	Hg	202
Rhodium	Rh	103	Thallium	Tl	205
Palladium	Pd	105	Lead	Pb	208
Silver	Ag	107	Bismuth	Bi	209
Cadmium	Cd	111	Thorium	Th	232
			Uranium	U	238

It must be noted that blood metal concentrations exceeding the RR reflect a high intake and/or exposure and are considered pathological. Table 2 compares the number and percentage of pathological blood values for the ASD and Control group. For the ASD group as well as the Control group an exceptionally high percentage of tests exceeded RR for the elements Aluminum, Barium, Manganese, Nickel and Lead. For Mercury, 16 percent of the ASD group showed pathological results, the Control group showed none.

Table 2: Number and percentage of blood tests showing pathological test results

	Number of tests	Al	Ba	Hg	Mn	Ni	Pb
95%ile RR mcg/l		**30**	**2.4**	**2**	**7.1**	**2**	**35**
Control group # of tests >RR	**7**	3	4	0	7	5	4
Control group % of tests >RR	7	***43***	***57***	***0***	***100***	***71***	***57***
ASD group # of tests >RR	**37**	18	28	6	34	30	30
ASD group % of tests >RR	**37**	***49***	***76***	***16***	***91***	***81***	***81***

Table 3 indicates that all 7 samples (100%) from the control group showed a urinary nickel excretion that exceeded the reference range, but only 74% of the ASD samples did. The percentage of urine samples exceeding the reference for Barium and Manganese was also higher in the Control group. However, the urinary excretion for Lead (Pb), and Mercury (Hg) was higher in the ASD group. For Aluminum no significant difference was noted between the groups.

Table 3: Percentage of urine tests with values exceeding upper reference range

	N	Al	Ba	Hg	Mn	Ni	Pb
95%ile RR		**40**	**5.7**	**1**	**4.5**	**3**	**5**
Control all % >RR	7	14	43	0	43	100	0
ASD all % >RR	39	13	33	2.6	26	74	10

Reference ranges in mcg/g creatinine.

Table 4 indicates that 28% of the Control group exceeded the hair RR for Aluminum, compared to 93% of the ASD group. For Barium and Lead, a higher percentage of tests exceeding RR was seen in the Control group, and for Manganese each group member showed unusually hair concentration. For Mercury, a greater percentage (49%) of the ASD group exceeded RR, compared to 22% of the Control group.

At present, state agencies do not provide reference ranges for metals in hair. Therefore, laboratories providing hair metal analysis develop their own ranges. Micro Trace Minerals has performed hair mineral analysis since 1984 and developed reference ranges on various populations, following standard laboratory procedures. The majority of hair samples used for statistics came from Germany and the US. Every 2-3 years, MTM re-evaluates statistical measurements and updates ranges if necessary, all according to laboratory regulations.

Table 5 to 10 shows group statistics, comparing mean concentration and standard deviation of blood, urine and hair.

Zinc status of both groups

Blood zinc levels reflect a deficiency state. In comparison, 27% of the ASD group showed

pathological blood values reflecting zinc deficiency, compared to 0% of the control group. Of the ASD males, 9 of 29 samples (or 31%) showed blood zinc levels below the reference range of 4mg/l, compared to one of 8 females (=12.5%).

We also noted that 16.7% of the ASD group showed a low urinary zinc excretion, compared to 5% of the Control group. Low urinary zinc excretion is not indicative of a nutritional deficiency.

When blood zinc levels are high and urinary zinc excretion is low, a disturbed zinc metabolism may be the cause. Increased tissue storage may result as was seen in both groups: 15% of the Control group and 18% of the ASD group.

Table 4: Percentage of hair sample results exceeding 95%ile reference range

	N	Al	Ba	Hg	Mn	Ni	Pb
95%ile RR		**8**	**2.65**	**0.3**	**0.5**	**0.85**	**3**
Control all							
% >RR	18	28	94	22	100	28	100
ASD all							
% >RR	43	93	67	49	100	33	86

Reference ranges are listed in mcg/g

Table 5: Group Statistics for Aluminum

VAR00001	N	Mean	Standard Deviation	Std. Error Mean
BLOOD: Control	7	**42.199**	**35.177**	**13.296**
ASD	39	35.123	18.699	3.033
URINE: Control	7	21.461	26.011	**9.831**
ASD	39	**30.722**	**47.011**	7.528
HAIR: Control	18	**29.930**	**16.696**	**3.935**
ASD	43	26.024	14.828	2.261

Table 6: Group Statistics for Barium

VAR00001	N	Mean	Standard Deviation	Std. Error Mean
BLOOD: Control	**7**	4.004	3.389	**1.281**
ASD	39	**4.914**	**4.274**	.693
URINE: Control	**7**	**8.759**	12.151	**4.593**
ASD	39	6.192	**12.798**	2.049
HAIR: Control	**18**	**19.449**	18.944	**4.465**
ASD	43	15.244	**19.470**	2.969

Table 7: Group Statistics for Lead

VAR00001	N	Mean	Standard Deviation	Std. Error Mean
BLOOD: Control	**7**	40.482	13.937	5.268
ASD	39	**60.368**	**53.247**	**8.638**
URINE: Control	**7**	1.193	.696	.263
ASD	39	**3.619**	**7.159**	**1.146**
HAIR-extreme values included				
Control	18	27.964	42.730	10.072
ASD	43	**100.389**	**584.125**	**89.078**
HAIR-extreme values excluded				
Control	**17**	**18.48**	**14.88**	**3.609**
ASD	43	11.14	8.14	1.241

Table 8: Group Statistics for Manganese

VAR00001	N	Mean	Standard Deviation	Std. Error Mean
BLOOD: Control	**7**	**51.301**	17.236	**6.556**
ASD	39	44.768	18.331	2.974
URINE: Control	**7**	**4.875**	3.633	**1.377**
ASD	39	4.305	3.490	.559
HAIR: Control	**18**	4.756	3.370	.794
ASD	43	**4.946**	**3.345**	**.510**

Table 9: Group Statistics for Mercury

VAR00001	N	Mean	Standard Deviation	Std. Error Mean
BLOOD: Control	**7**	**1.257**	**.375**	**.142**
ASD	38	1.218	.779	.126
URINE: Control	**7**	.112	.101	.038
ASD	39	**.272**	**.381**	**2.245**
HAIR: Control	**18**	.181	.112	.026
ASD	43	**2.723**	**14.724**	**2.245**

Table 10: Group Statistics for Nickel

VAR00001	N	Mean	Standard Deviation	Std. Error Mean
BLOOD: Control	7	2.385	.872	.330
ASD	39	**3.449**	**2.414**	**.392**
URINE: Control	7	6.705	2.446	.925
ASD	39	**10.915**	**18.812**	**3.012**
HAIR: Control	18	.830	.562	.132
ASD	43	.839	.673	.103

Discussion:

Medical literature suggests that the elements tested (Al, Ba, Pb, Mn, Hg) with the possible exception of Ni, affect neurological health. We statistically compared mean and standard deviation of the various diagnostics performed at Micro Trace Minerals Laboratories as seen in Table 5 through 10.

Aluminium: by evaluating the mean Al blood concentration and standard deviation of the group statistics (Table 5), we noted that both study groups are similarly exposed with a somewhat higher mean Al blood concentration of 42.199mcg/l in the Control group compared to 35.123 mcg/l in the ASD group. It may be of interest that an independent statistical evaluation of 1500 blood aluminum tests from the MTM 2017 data base showed a mean value of 26mcg/l with a standard deviation of 32mcg/l.

Our urine data indicates that the ASD group shows a higher mean excretion value than the Control group. The RR for urine is 40mcg/g creatinine, and 16% of the ASD samples show urine Al concentrations above that RR, compared to 14% of the Controls. The highest percentage of samples exceeding the RR was obtained from the Control group females (33%), the lowest (0%) from the females of the ASD group. From the males of the control group, zero percent exceeded the RR, compared to 16.7% of the ASD males.

The group statistics for hair shows a similar mean hair concentration for both groups, indicating that the long-term aluminum exposure is similar.

Barium: The reference range for Ba in blood is 2.4mcg/l, with 28% of the Control group and 76% of the ASD group exceeding that range. The group statistics show a similar mean blood concentration (Table 6). The urinary output is higher in the Control group, suggesting a more effective renal excretion, which would lead to a decreased tissue storage; however, our group statistics show a similar mean concentration in both groups, with a slightly higher mean in the Control group. For this we have no explanation.

Lead: As early as 1996, Millichap reported that lead poisoning was evident in 75% of children with pervasive developmental disorders, including ASD, compared to 23% of randomly selected children without ASD. (Millichap, 1996) Rossignol et al stated that environmental toxins such as lead (Pb) could cause neurodevelopmental disorders such as ASD, because the developing brain is more susceptible to injury from toxicants than the adult brain. (Rossignol, 2016).

The blood reference range as given by the German Environmental Protection Agency (Umweltbundesamt) for blood lead of children is 35mcg/l (see Table 2). MTM's internal statistical evaluation on 1528 blood lead tests from Western people shows a mean lead value of 14.96mcg/l with a standard deviation of 12.78mcg/l. The ASD group showed a mean level of 60.37mgPb/l, compared to a mean of 40.48mcgPb/l for the control group.

The urine lead data from the ASD group shows a higher mean excretion value of 3.62mcg/g creatinine than the Control group (1.19mcg/g creatinine). When compared to a mean urinary lead value of 0.66mcg/g creatinine (Stdev 3.92mcg/g crea) derived from over 10000 unprovoked urine samples from the MTM database, both Nigerian test groups exceed urinary excretion values of a European population.

The RR for Pb in hair is 3PPM. We did note an extreme concentration of 3841mcgPb/g in one of the samples from the ASD group, which explains the high standard deviation listed in Table 7. This extreme hair lead concentration was validated through multiple testing. Our investigation indicates that this hair sample is from a 7year old ASD male on whom a hair straightener was used. The product description did not list Pb as an ingredient. After excluding the extreme values from both groups, Table 7 shows that the ASD group statistics seems less affected by a long-term lead burden, but we are

hesitant to make such conclusion. In MTM's near 50-year experience with hair metal testing, extreme hair lead concentrations (3841mcgPb/g hair) as seen in the ASD child are generally due to external hair treatment such as black hair colorings. The information provided by our Nigerian provider scientists potentially rules out lead hair treatment. Since this 7year old child also showed a high blood lead level of 69.69 mcg/l compared to the German RR for children of 35mcg/l, excessive environmental exposure may be the cause of the child's lead levels. However, the child's urinary output was low, 1.9mcg/g creatinine compared to a RR of 5mcg/l. This leads us to assume that the high exposure as seen in the child's blood, followed by a low urinary output may be the cause of the child's excessive burden as reflected in hair.

We also noted another sample with an unusually high lead hair concentration of 189 mcg/g in the Control group. This hair sample came from a 13year-old male. According to our investigator, no chemical hair treatment was used. Unfortunately, due to shipping problems, we did not have a blood or urine sample for comparison.

However, with or without the inclusion of these 2 extreme lead hair concentrations, both groups show an unusual lead burden.

Manganese: The essential trace element Manganese is neurotoxic in excess, with mechanisms reflecting lead toxicity. Our research clearly demonstrates that 100% of the ASD *and* 100% the Control group exceed hair reference ranges for manganese. This indicates that all participants are affected by short *and* long-term exposure. In Table 8, we compare mean values for blood, urine and hair of both groups. The mean blood manganese concentration of both groups indicates an excessive immediate manganese exposure, leading to an excessive chronic manganese burden. Males and females of both groups were equally affected.

Mercury in any form is toxic to humans, and mercury exposure from ingestion, inhalation or absorption through the skin affects the neurological, gastrointestinal, and renal organ system.

Our data demonstrates that long-term mercury exposure is a greater problem for autistic than healthy people. Hair analysis data exceeding the reference range were more than twice as high in the ASD than the control group. Blood levels, reflecting immediate exposure and uptake, also differed: 60% of the ASD samples exceed the RR compared to 9% for the Control group. Since the percentage of urinary mercury excretion did not significantly exceed the respective reference range for either group, mercury exposure is a considerable problem, leading to tissue accumulation, and indeed, the group statistic shows a significantly higher chronic mercury burden for the ASD group. For the ASD group, the mean Hg hair concentration is 2.72mcg/g with a high standard deviation of 14.72mcg/g hair. For the Control group, the mean concentration is 0.181mcgHg/g hair with a standard deviation of 0.112. This demonstrates that the long-term chronic Hg exposure is considerably less in the Control group.

We noticed one extreme hair mercury value of 96.75 mcg/g from an 8-year old ASD boy. This value was confirmed through multiple testing. (Note: to our knowledge, hair cosmetics do not contain mercury, thus hair cosmetics were ruled out as a source of external contamination.) The boy's blood or urine mercury concentration were inconspicuous. The urine Hg-concentration was 0.16mcg/g crea, the blood mercury concentration of 0.69mcg/l was below the blood reference range of 1mcg/l. We can only assume that the boy experienced a significant mercury overexposure in the past, which may have contributed to the onset of ASD.

Nickel: An immediate nickel exposure is seen in the mean blood concentration of both groups, with the ASD group showing a higher blood

nickel mean of 3.449mcg/l, compared to a mean of 2.385mcg/l for the control group. (Table 10)

The hair group statistic demonstrates that the chronic nickel exposure of both groups is similar, and our urine data supports official statements that most of the absorbed nickel is excreted in urine, regardless of the route and type of exposure. The ASD Group, which showed higher blood levels excreted more nickel.

Conclusion

The findings of this study indicate that both study groups, the healthy and ASD children of the Niger Delta, show an unusually high metal burden. A low zinc status is only prevalent in the ASD group, males were more affected than females, which confirms the 2011 study by Yasuda *et al*.

The metal concentration in blood reflects daily exposure levels. In both groups, between 43% to 100% of tests showed pathological blood values for the elements Aluminum, Barium, Manganese, Nickel and Lead. No pathological blood mercury values were seen in the healthy control group, but 16% of the ASD children's blood results exceeded the reference range as provided by German government officials.

Urinary excretion levels normally rise after immediate exposure. For Lead and Nickel, the mean urinary excretion supported mean blood values, but otherwise, renal excretion did not provide clear data.

The metal concentration in hair tissue reflects a metal burden that accumulated over time. It is no surprise that both groups, the healthy and ASD children of the Niger Delta, show high hair metal concentration. The immediate and long-term Manganese exposure is excessive and needs attention. Similarly, lead overexposure is seen in both groups. The long-term burden for Aluminum and Mercury was significantly higher in the ASD group, whereas the Control Group showed a higher chronic burden of Barium.

We conclude that the high metal burden found in the children of both groups is most likely the result of environmental exposure. It is surprising that this unusually high exposure to neurotoxic elements such as Manganese and Lead does not seem to affect the neurological health of *all* children living in the Niger Delta. If Rossignol's hypothesis is correct and overexposure to lead does cause neurodevelopmental disorders, why does the healthy group of Nigerian children with a high metal burden not show signs of ASD? Are they affected by other health problems related to lead or manganese or mercury toxicity?

Our research project focused on environmental metal exposure as a notable cause of ASD, and our data suggest that exposure to neurotoxic metals in combination with zinc deficiency deserves attention. We are also faced with the question why metal-burdened children who live under similar conditions than ASD children, remain neurologically unaffected.

Regarding both groups, we suggest frequent attention to the children's metal status. Metal detoxification treatments should benefit both groups. In addition, we suggest that children living in the Niger Delta receive frequent health checks, including blood and hair analysis, and because zinc is important for growth and the body's detoxification system, a yearly evaluation of their zinc status is recommended, particularly for the neurologically affected.

Conflict of Interest: None

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