The use of urinary porphyrins analysis in autism

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Abstract

The analysis of urinary porphyrins promises to provide substantial insight into treating autism. Previous methods to determine body burden of toxic metals relied on a chelation challenge. However, the urine porphyrins test is more effective than a chelation challenge in determining toxic metal body burden because it measures the effects of those metals on the body. Specifically, certain metals, particularly toxic metals such as mercury, lead, and arsenic, inhibit different enzymes in the porphyrin pathway for the synthesis of heme and, thus, cause different and specific porphyrin patterns (or "profiles") in the urine, the analysis of which can help determine which metal is involved, and to what degree. The higher the toxic metal exposure and body burden, the higher the porphyrins are elevated in the urine. In humans, certain urinary porphyrin profiles appear to directly reflect mercury body burden and neurobehavioral deficits.

This article reviews one recent prospective study of 115 children with autism who demonstrated porphyrinuria when compared to 119 control children, including a mean increase of 2.6-fold ($p < 0.001$) in coproporphyrin. The elevation in coproporphyrin also correlated with the severity of autism and was consistent with mercury exposure and burden. A subgroup of these autistic children underwent oral chelation therapy with meso-2,3-dimercaptosuccinic acid (DMSA), which resulted in a significant reduction in mean urinary coproporphyrin and precoproporphyrin ($p = 0.002$). Another prospective study on 37 autistic patients confirmed that the severity of autism was directly correlated to the degree of porphyrinuria. The interpretation of the urinary porphyrin profile is reviewed, as are important differences found in males versus females.

Introduction

The analysis of urinary porphyrins promises to provide substantial insight into treating autism. Before diving into porphyrins, let's start with a quick overview of what happens when toxic metals, such as mercury and lead, arrive in the body. When we are exposed to mercury or lead, whatever is not rapidly excreted circulates in our blood for about a month or so [1] until it is eventually incorporated into various body tissues. Mercury is deposited in the kidney, liver, heart, glands, and brain. Lead is typically deposited in the brain and bone. So if you are poisoned by mercury or lead during a single bolus exposure event, and we then measure a blood or urinary level several months later, those levels will be essentially zero [1].

Prior to the advent of urinary porphyrins analysis, we would perform a chelation challenge where we would administer to the patient a chelator that has a negative charge (an anionic chelator) and an affinity for multiply charged positive ions (cations), and it would find these cations, including metal ions such as mercury (II) and lead (II) ions, bind to some of them ("complexing" them), and then the body would be able to excrete those complexed metal ions in the chelation-challenged patient's urine. We would then measure the level of the complexed metal ions in the urine. However, if the metals were tightly bound deep in body tissues, then the chelator typically would not pick them up easily and, thus, we would not see an elevated level of metals in the post-challenge urine in such instances. If we saw a lot of metals in the urine after the chelation challenge then that was great; however, for the reason stated often we did not even in cases where it was clear the child had

had significant documented exposures. So the chelation challenge often did not let us estimate the body burden of metals in a particular individual, and sometimes missed certain metals completely.

In my opinion, the urine porphyrin profile analysis test is more effective than a chelation challenge in determining toxic metal body burden because it measures the effects of those metals on the body as follows.

Porphyrins belong a special class of uniquely-structured and uniquely-sized polycyclic compounds having a central opening (cavity) that living organisms can use to surround and bind nearly any metal ion. Thus, porphyrins have diverse biological functions (see, e.g., http://en.wikipedia.org/ wiki/Porphyrin or http://www.answers.com/topic/porphyrin). For example, heme proteins (which contain iron porphyrins) are very common in nature and serve many roles, including binding, transport, and storage of oxygen and carbon dioxide (myoglobin and hemoglobin), electron transport (cytochromes b and c), oxygen activation and utilization (cytochrome oxidase), and managing and using hydrogen peroxide (peroxidases and catalases). Other porphyrins play a role in detoxifying certain chemicals such as pesticides and other toxins (P450 enzymes). Related porphyrins include the chlorophylls (which have a central magnesium ion) and the pheophytins (which are metal free), which are respectively used by plants and bacteria for photosynthesis. Another common porphyrin is vitamin B-12, which: a) has a central cobalt ion and b) is found in both bacteria and animals. In living systems, zinc-, copper-, and nickel- containing porphyrins are also known, and many other metals can be bound in a porphyrin's central cavity when living systems are exposed to them.

Porphyrins are involved in the production of heme, which is a necessary ingredient of the P450 enzymes that are critical for detoxification of chemicals and toxins, including pesticides. Heme is also necessary to remove beta-amyloid plaques from the brain [2]. In a recent study, children with severe autism secreted 2-3 times more beta-amyloid precursor protein than children without autism [3]. To generate heme, via specific enzymes, the body normally converts one species of heme porphyrin into another, in a sequential metabolic order or pathway. Yet, certain metals, particularly toxic metals such as mercury, lead, and arsenic, will inhibit different enzymes of the heme porphyrin pathway and will thus cause different and specific porphyrin patterns (or "profiles") in the urine, the analysis of which can help us determine which metal is involved, and to what degree [4]. For example, biphenyls, dioxins, and aluminum inhibit uroporphyrin decarboxylase, which is an early enzyme in the porphyrin pathway, while mercury inhibits coproporphyrinogen oxidase, which is a later enzyme, and lead inhibits both coproporphyrinogen oxidase and aminolevulinic acid dehydratase.

In short, if a heme porphyrin enzyme is inhibited, the corresponding porphyrin will not be able to convert to the next in the metabolic pathway, as would normally occur if the enzyme were uninhibited. Thus, that affected porphyrin will build up in the body, and more of it will be excreted in the urine. The more types and/or quantities of inhibiting metals that are present in the body, the higher the inhibition and the more types and/or quantities of affected heme porphyrins will appear in the urine (and in the analysis of that urine, which we call the "urinary porphyrin profile"). Wherever the inhibiting metal is located in the body, whether deep in the tissue or superficial, the porphyrin-converting enzyme inhibition will likely take place and the corresponding porphyrin will appear in the urine. Consequently, by measuring the effects of these particular metals in the porphyrin pathway, we can indirectly determine which metals are present in the body, and, in general, at what levels.

Urinary porphyrins studies

The study of porphyrins in human diseases was pioneered by Dr. Jim Woods out of Seattle, who began this work roughly 30 years ago. At the Seattle 2006 Fall DAN! Conference, Dr. Bradstreet and I met with Dr. Woods, and he spoke during Dr. Bradstreet's lecture. At ICDRC, we are currently collaborating on some studies with him, so stay tuned as we think the results will be exciting and surprising to many people.

Many studies have demonstrated a link between exposures to toxic substances and porphyrin problems. For example, a study by Fowler showed that the metabolic pathway for the heme porphyrin is "highly sensitive to inhibition" by metals such as lead, mercury, and arsenic [5]. In animal studies, Miller demonstrated that mercury exposure causes an excretion of excessive porphyrins in the urine (called porphyrinuria), including elevations in urinary excretion of penta-carboxyporphyrin, coproporphyrin, and precoproporphyrin, compared to the unexposed control animals [6]. In a rat study, Woods demonstrated that porphyrinuria caused by methylmercury first occurred at doses that did not produce obvious neurological problems [7]. Gonzalez-Ramirez later replicated these findings in humans

exposed to mercury vapor [8]. Heyer showed that the urinary concentration of porphyrins can serve as "a biomarker of prolonged exposure to all forms of mercury." Furthermore, the higher the mercury exposure, the higher the porphyrins were elevated in the urine [9]. Woods demonstrated that in humans, certain urinary porphyrin profiles directly reflect mercury body burden and neurobehavioral deficits [Woods, 1996]. Echeverria found declines in "neurobehavioral performance" at mercury concentrations less than 4 μg/L (0.004 ppm) in the urine, which is an extremely low level and approaches what is found in the general population today [10].

The key to the urinary porphyrin profile test is that it can help estimate the body burden of toxic metals, which can otherwise be difficult to determine. Previous studies demonstrate that some chronic symptoms (those present for longer than 1 year) caused by mercury can remain undiscovered when evaluations only rely on prechelation urinary measures of mercury exposure [11]. This is because urinary measures of mercury prior to chelation reflect only recent exposure (over the previous 2-3 months) [9]. In other words, the amount of mercury present in the urine before administration of a chelator does not correlate with the body burden of mercury. However, the amount of mercury present in the urine after a chelation challenge with dimercapto-propanesulfonate (DMPS) is, in many cases, a measure of mercury exposure [8] and estimates the body burden of mercury [11,12]. Animal studies demonstrate that treatment with DMPS significantly decreases the kidney concentration of mercury and increases urinary mercury excretion. In addition, DMPS treatment decreases both kidney and urinary porphyrin concentrations, which are highly correlated (r ~ 0.9) with the decrease in mercury concentration in the kidney. Therefore, urinary porphyrins are a biomarker of the renal mercury content [12]. In addition, when autistic children have an increased body burden of toxic metals compared to control children, we would expect more toxic metal urinary excretion after a chelator dose [10].

Genetics and porphyrins

Several studies demonstrate a link between specific genetic variations and certain porphyrin problems. For example, Woods recently described a polymorphism in exon 4 (N272H) of the gene that encodes for the production of the heme pathway enzyme called coproporphyrinogen oxidase (also known as CPOX-4). The homozygous genotype occurs in approximately 3% of the population and the authors hypothesized that a CPOX-4 polymorphism might increase the risk of neurotoxicity upon exposure to mercury [13]. In another study by Heyer, people who were homozygous recessive for the CPOX-4 polymorphism had evidence of increased neurotoxicity to dental mercury exposure when compared to the remaining population. In other words, the study was "suggestive that the CPOX4 polymorphism could increase susceptibility to mercury-related neurobehavioral effects" and therefore the CPOX4 polymorphism "might serve as a biomarker of susceptibility to mercury toxicity" [9]. This supports the idea of a genetic susceptibility to mercury toxicity, something that many of us have stated about autism for some time now [14, 15]. Heyer and Echeverria also demonstrated a genetic susceptibility to mercury toxicity in

humans exposed to chronic low-level mercury in which a polymorphism in brain-derived neurotrophic factor (BDNF) was associated with increased self-reported mood and symptom scores (p = 0.0039) [16] and changes in cognitive and motor function [11]. In other words, people who are homozygous recessive for the CPOX-4 and BDNF genes appear to be more susceptible to mercury toxicity compared to the general population. Interestingly, problems in BDNF are present in some autistic individuals [17]. Further study is needed to determine if children with autism are more likely to have polymorphisms in these 2 genes compared to the general population. Since Woods showed that the rate of CPOX-4 polymorphism is approximately 3% in the general population [13] and Echeverria showed that the BDNF polymorphism occurs in approximately 4-5% [11], large-scale population studies might miss increased susceptibility to mercury toxicity in this small portion of people. Any problems found in 1 or 2 people during large studies tend to be averaged out because of the large number of people involved (in studies these people are usually called "outliers" and are excluded from analysis). The CPOX-4 gene defect is present in about 3% of population, but might be higher in autism. As an aside, Woods has shown that people with low glutathione levels have more porphyrins in the urine [18] because there is less glutathione available to bind to the metals and prevent toxicity. Of course, we know that most children with autism have documented lower levels of glutathione [19].

Porphyrinuria and autism

One recent prospective study of 115 children with autism demonstrated porphyrinuria when compared to 119 control children [20]. In this study, 53% of the autistic children had elevated urinary porphyrin levels. This study enrolled 115 children with autism, 63 with PDD-NOS, and 88 children with other neurological disorders such as Asperger disorder, attention deficit, hyperactivity, epilepsy, and cerebral palsy.

When compared to the control group, children with autism had a mean increase of 2.6-fold (p < 0.001) in coproporphyrin (which I refer to as "copro"). This elevation persisted when normalized to urinary levels of creatinine. The elevation in coproporphyrin also correlated with the severity of autism. The autistic children also had significantly higher levels of mean urinary precoproporphyrin ("preco", p < 0.001), pentacarboxyporphyrin ("penta", p < 0.001), and hexacarboxyporphyrin ("hexa", p <0.002). There was also a slight elevation of uroporphyrin levels in the children with autism, which could be consistent with xenobiotic (chemicals or pesticides) exposure, but the difference was not significant versus the control group. A subgroup of these autistic children underwent oral chelation therapy with meso-2,3-dimercaptosuccinic acid (DMSA), which resulted in a significant reduction in mean urinary coproporphyrin and precoproporphyrin (p = 0.002), indicating that the urinary porphyrin elevation was not genetic in nature but due to the toxic metals removed.

Children with autism and seizures had even higher levels of mean coproporphyrin compared to children with autism without seizures. This is not surprising because seizures can be induced by certain toxic metals [21], especially mercury [22], and Dr.

Nataf showed that mean copro levels in children with seizures were higher than in children with autism [20].

Another prospective study on 37 autistic patients confirmed that the severity of autism was directly correlated to the degree of porphyrinuria. Non-chelated children with autism had more porphyrins present in the urine compared to children with PDD, Asperger syndrome, or control children. Control children were neurotypical and matched according to age, sex, and race. Non-chelated autistic children also had higher levels of mean coproporphyrin (1.7-fold) when compared to chelated autistic children (p < 0.05), and almost 3-fold higher when compared to the control group (p < 0.05) [23]. This is also what we are finding clinically at ICRDC—in general, the more severe the autism in a child, the higher the urinary porphyrin levels.

In some studies, elevated porphyrins appear to directly cause toxicity. Ruscito showed that some porphyrins interact with the binding site for benzodiazepines on the gamma-aminobutyric acid (GABA$_A$) receptor and causes synaptic transmission inhibition, much like diazepam [24]. Also, some porphyrins, if the levels are high enough, appear to directly cause neurotoxicity [25].

Applying the urinary porphyrins test

So, how do I use the porphyrin test in autistic children? The history is always the most important place to begin. I first look at the metal exposure history of the child. Since 1 out of 6 children in the United States is born with blood mercury levels high enough to impair neurological development [26], I can expect that some children will have this as a main exposure. The amount of fish mom ate and the number of maternal amalgams present during pregnancy are important because both of these correlate with the amount of mercury present in the fetal blood and brain in utero [27]. In many studies, the fetal blood level of mercury was shown to be approximately twice that of maternal blood [28, 29]. Furthermore, many children are exposed to lead during pregnancy as maternal bone is broken down to release calcium and thus also lead [30]. Other exposure history in some children includes immunizations containing thimerosal, fish consumption, lead in the environment (which is practically ubiquitous), antimony from flame retardant bedding and pajamas, arsenic from chicken and treated wood, pesticide exposure, etc.

So, before ordering a urinary porphyrin profile, I have an idea of the exposure history and what the porphyrin test might show. However, I don't know what amount of the toxic metals each child is going to retain after exposure. What we think is occurring in autism is a reduced ability to excrete certain metals and toxins [14, 15]. In many children, I have a general idea that the porphyrin profile will be elevated but not how high it will be. However, sometimes I am surprised by the profile, or wrong, so ordering the profile is important. I do find that the severity of elevation on the porphyrin test helps me to know how aggressive to be with chelation.

As an aside, if your child has a fairly high toxic metal exposure history but a porphyrin profile that is very low, then it may be a false-negative. A false negative can occur if the porphyrins become oxidized by substances or damaged by light. Oxidation of porphyrins can occur from the breakdown products from

supplements that are excreted in the urine and many of our children are on high doses of nutritional supplements. Therefore, a second-morning urine is more helpful in that case, as the breakdown products from supplements are concentrated in the urine throughout the night. Dr. Woods and his colleagues have also found that a second-morning urine is more accurate in general [31], and this is usually what we recommend. However, a second-morning urine can also lead to a low creatinine level in the urine, which can affect the interpretation of the lab. Dr. Nataf has indicated to us that the porphyrin test is accurate as long as: a) he can test the "stabilized" sample within about 3 weeks and b) that sample is protected from exposure to light. I generally recommend collecting the porphyrins in a glass or paper vessel if possible to minimize contamination from other types of containers. The sample can be shipped to France by U.S. postal mail, costing typically less than two dollars.

False-negative results appear to be more common in women. In some studies, the excretion of porphyrins in the urine is sporadic in women but not as much in men (we assume the same holds true for children, but these studies were done in adults). The excretion of porphyrins in most people has a diurnal variation [6], which means that it has peaks and valleys throughout the day. In men the excretion of porphyrins in the urine is fairly constant, and Woods has shown that a spot urine correlates well with 24-hour urine porphyrin levels in men. However, in women, the excretion of porphyrins is more variable throughout the day and therefore a spot urine does not always correlate well with 24-hour urine porphyrin levels [32]. In girls, we still do a spot urine test (mainly because a 24-hour urine test is not always feasible), but if the porphyrin levels are low (especially if exposure levels are high), then we strongly consider repeating the porphyrin sample or collecting a 24-hour urine porphyrin sample if possible. Since porphyrin excretion varies throughout the day in most people, when repeating porphyrins to assess treatment outcomes, it is important to collect the urine at the same time of day as the previous sample in order to make comparisons more reliable. One study demonstrated that the best time to collect a urine sample for porphyrins was mid-morning as this correlated best with 24-hour urinary porphyrin levels [31]. Therefore, I generally recommend performing a second-morning urine collection.

Porphyrin excretion is representative of the toxic metals present in the kidney. Therefore, it does not necessarily represent what metals are present in the brain or in what quantities. Currently, short of a brain biopsy, we do not have a simple hospital (e.g., MRI) or lab measurement for types or amounts of toxic metals that are present in the brain. However, we presume that the levels of the toxic metals present in the kidney are reflective of what is present elsewhere in the body, including the brain. In multiple studies, the level of porphyrins in the urine correlates with the level of metals present in the kidney upon autopsy. Furthermore, as urinary porphyrin levels decrease with chelation, the level of mercury present in the kidney drops in proportion to the decrease in urinary porphyrins [12]

So which lab should you use to measure urinary porphyrins? I prefer and use Dr. Nataf's lab in France. His group was the first to describe the use of porphyrins in the urine of autistic children. Based upon his study, we know the mean values of urinary porphyrins in control children and in children with au-

tism, PDD, Asperger syndrome, seizure disorder, etc. These values are helpful because they provide reference ranges that we can use in interpreting the test results. I tend not to use LabCorp, Quest, and other U.S. labs because the norms for many of these labs are typically for adult healthy patients and not for autistic children. Furthermore, many of the U.S. labs use the porphyrin test to rule out genetic porphyrias where massive amounts of porphyrins are present in the urine. Thus, they can sometimes miss the small elevations that we find in autistic children because their lab machinery is not calibrated properly. In addition, the units of measurement used in Nataf's lab and the U.S. labs are different, and need to be converted to nanomoles per gram of urinary creatinine (Nataf's unit of measure) to compare measured values to the reference range in autistic children as described by Dr. Nataf (this can be quite tedious). Furthermore, Dr. Nataf's lab and Dr. Wood's lab in Seattle are the only labs that I know of in the world that currently report preco levels (Dr. Wood's lab only does it for research purposes). This marker is extremely important as discussed below.

When interpreting urinary porphyrin levels, I first look at the amount of creatinine present in the urine. This is typically listed on the very bottom of the first page of the Nataf report. I like to see around 1000 mg/l. If it is less than 1000 mg/l, then the levels of porphyrins reported as present in the urine could be falsely elevated. If it is more than 1000 mg/l, then the level of porphyrins reported could be underestimated. However, this is not a hard and fast rule because children with autism often have lower creatinine levels than neurotypical children [33], which makes interpretation of all this somewhat tricky. Suffice it to say that if the urinary creatinine is near 1000 mg/l plus or minus 200-300 mg/l or so, I am generally happy with that. If the urinary creatinine is out of this range, then we have to be careful with the interpretation, and sometimes it is worth repeating the sample. However, often times the porphyrin values are so elevated, that even if the urine is too dilute and the creatinine is low, the urinary porphyrin values are still quite elevated even after correction.

Next, I look at the copro level. Typically, this is elevated with respect to the listed reference range due to exposure to mercury, lead, and/or arsenic. The copro level is a good marker of *overall* toxic metal burden in the kidney. Then, I look at the preco level. Since the only thing that is known to elevate the preco value is mercury [13], preco is a marker of mercury exposure and nothing else. Preco is called an atypical urinary porphyrin because it is not normally present in the urine, and is only found with elevated mercury levels in the body. If the copro and preco are elevated by about the same percentage over their respective reference ranges, this is usually a good reflection of mercury. If the copro is more elevated in comparison to the percentage of elevation in preco, then usually another metal is heavily at play here, typically lead or arsenic. If the percentage of increase in preco is more than the percentage increase in copro, then mercury is predominant. Next, I look at penta, which is elevated with mercury exposure, but is typically less elevated than preco because it is upstream of the enzyme inhibited by mercury in the porphyrin pathway. Finally, I look at the heptacarboxyporphyrin ("hepta") and uroporphyrins ("uro") levels. These are typically elevated over their reference ranges due to xenobiotic exposure such as PCB's, pesticides, and other

toxic chemicals, such as dioxins. However, arsenic and aluminum can also cause these to be elevated. So with elevated uro-porphyrins, it can be difficult to tell if the elevation is from toxic chemicals, arsenic, and/or aluminum.

For Dr. Nataf's lab, he also lists several ratios that can be helpful in interpreting the lab results, along with respective reference ranges for each ratio. For example, a preco/uro ratio is provided. Since uro is not elevated by mercury, but preco is, the ratio of these two is helpful to confirm that the preco elevation is real and not a false positive result. Also listed (sometimes on the second page) is a preco/copro ratio. An elevated preco/copro ratio can tell us that not only is mercury present, but that it is the predominant metal. A preco/copro ratio that is lower than the reference range (but copro is still elevated) indicates that another toxic metal like lead or arsenic is predominant. The final ratio listed is an uro/copro ratio. An elevated ratio of uro/copro is consistent with xenobiotics, arsenic, or aluminum exposure and confirms that the elevation in uro is not a false positive. As an aside, we know that some children with autism have genetic defects in the paraoxonase gene and therefore cannot properly detoxify pesticides [34]. It must be noted that many times, the children we evaluate were exposed to many different toxic metals and chemicals, including mercury, lead, aluminum, arsenic, pesticides, PCB's, and others. Therefore, the ratios can be somewhat tricky to interpret as we have to figure out how each metal or toxin will affect the overall porphyrin profile.

Interpreting mercury exposure on the porphyrin test is relatively easy by looking at the preco level. However, lead is a little more difficult to interpret. Generally with lead exposure, the profile will reflect a high copro level, but this elevation can also be from mercury or arsenic. An exposure pattern (which I see on occasion), which is consistent with lead only (and not mercury), is a high copro and a much lower preco level. I think that it is generally important to measure a blood lead level to exclude active exposure to lead, as lead poisoning is known to mimic autism [35]. In fact, I have discovered a few children who were getting active lead exposure. This led to their autistic symptoms, which improved when the lead exposure was removed. Some unusual lead exposures that I have seen are from physical therapy vests made of lead or old bathtubs with cracks in them that release lead. Arsenic and aluminum burdens are very difficult to interpret based upon the porphyrins test because the results overlap with other toxins. As an aside, I have started collecting some porphyrin results on the parents of autistic children, and am finding high porphyrin levels in some.

Once a high porphyrin level is established, I find it helpful to then sometimes perform a chelation challenge and obtain a "provoked urine" toxic-metal test when the type of metal present is still in question. Using different chelators can sometimes be helpful to know how a child will respond to each. I prefer suppositories or IV's because I generally see the best results with these. In addition, I am doing a lot less chelation challenge tests now because of the porphyrin test. If the preco is fairly elevated, then I know I need to use a chelator that will remove mercury. So typically I will start with DMSA or DMPS, but I do also use a lot of calcium-disodium-ethylenediamine-tetraacetate acid (EDTA), as many of the children I am seeing have evidence of unrecognized elevated lead levels. Sometimes,

this means I am using DMPS and EDTA together. Of course, the choice of chelator(s) is individualized to each child.

Recently, some people have expressed concern that, based upon genomic testing, sulfur-containing chelators may not be tolerated by some autistic children. However, based upon my review of the literature, a very consistent finding in most children with autism is a deficiency in sulphates, which can lead to a decreased ability to properly metabolize toxins [36]. If an increase in sulphates is suspected based upon genomics, it is quite easy to measure a blood sulphate level and a urinary sulphate level, and determine if there is truly an increase. If a child has very high urinary porphyrin levels (i.e. copro > 400 or preco > 25), it is my opinion that removing the toxic metals without using DMPS or DMSA is going to be difficult. Furthermore, an IV or suppository form of chelation is sometimes required.

We might be able to use the porphyrin test to determine when to stop chelation. However, a recent study on using chelation in rats to remove lead from the brain showed that lead was still being removed from the brain even after the measured blood lead level declined to zero [37]. Therefore, this *might* mean that we want to continue chelation for a period of time even after the levels of abnormal porphyrins or metals in the urine toxic-metal tests drop below normal levels.

Nataf was able to use DMSA chelation orally to bring the porphyrin levels to normal [20], so we might be able to use the urinary porphyrin test sequentially to help us determine when we are nearing the end of chelation. However, we are still in the process of evaluating this. In general, for most children, I can say that the urinary porphyrin levels are coming down with chelation over time, and autistic symptoms in many children are improving.

So, is the urinary porphyrin test reliable? Dr. Woods has done research on this test for about 30 years and has shown that it is very reliable, especially for mercury exposure. Recent studies have shown the amounts of porphyrins present in the urine prior to commencing chelation correlate well with the severity of the autism [23]. This is also our experience at ICDRC. I personally feel that the porphyrin test will revolutionize the way we treat toxic metals in autism. Stay tuned as we are currently involved in several studies looking into this issue further.

References

[1] Clarkson TW. The three modern faces of mercury. Environ Health Perspect 2002;110(S1):11–23.

[2] Atamna H, Boyle K. Amyloid-beta peptide binds with heme to form a peroxidase: Relationship to the cytopathologies of Alzheimer's disease. PNAS 2006;103;3381–6.

[3] Sokol DK, Chen D, Farlow MR, Dunn DW, Maloney B, Zimmer JA, Lahiri DK. High levels of Alzheimer beta-amyloid precursor protein (APP) in children with severely autistic behavior and aggression. J Child Neurol 2006 Jun;21(6):444–9.

[4] Woods JS. Altered porphyrin metabolism as a biomarker of mercury exposure and toxicity. Can J Physiol Pharmacol 1996;74(2):210–5.

[5] Fowler BA. Porphyrinurias induced by mercury and other metals. Toxicol Sci 2001;61(2):197–8.

[6] Miller DM, Woods JS. Redox activities of mercury-thiol complexes: implications for mercury-induced porphyria and toxicity. Chem Biol Interact 1993;88(1):23–35.

[7] Woods JS, Fowler BA. Renal porphyrinuria during chronic methyl mercury exposure. J Lab Clin Med 1977;90(2):266–72.

[8] Gonzalez-Ramirez D, Maiorino RM, Zuniga-Charles M, Xu Z, Hurlbut KM, Junco-Munoz P, Aposhian MM, Dart RC, Diaz Gama JH, Echeverria D, et al. Sodium 2,3-dimercaptopropane-1-sulfonate challenge test for mercury in humans: II. Urinary mercury, porphyrins and neurobehavioral changes of dental workers in Monterrey, Mexico. J Pharmacol Exp Ther 1995;272(1):264–74.

[9] Heyer NJ, Bittner AC Jr, Echeverria D, Woods JS. A cascade analysis of the interaction of mercury and coproporphyrinogen oxidase (CPOX) polymorphism on the heme biosynthetic pathway and porphyrin production. Toxicol Lett 2006;161(2):159–66.

[10] Echeverria D, Woods JS, Heyer NJ, Rohlman D, Farin FM, Li T, Garabedian CE. The association between a genetic polymorphism of coproporphyrinogen oxidase, dental mercury exposure and neurobehavioral response in humans. Neurotoxicol Teratol 2006; 28(1):39–48.

[11] Echeverria D, Woods JS, Heyer NJ, Rohlman DS, Farin FM, Bittner AC Jr., Li T, Garabedian C. Chronic low-level mercury exposure, BDNF polymorphism, and associations with cognitive and motor function. Neurotoxicology and Teratology 2005;27:781–96.

[12] Pingree SD, Simmonds PL, Rummel KT, Woods JS. Quantitative evaluation of urinary porphyrins as a measure of kidney mercury content and mercury body burden during prolonged methylmercury exposure in rats. Toxicol Sci 2001;61(2):234–40.

[13] Woods JS, Echeverria D, Heyer NJ, Simmonds PL, Wilkerson J, Farin FM. The association between genetic polymorphisms of coproporphyrinogen oxidase and an atypical porphyrinogenic response to mercury exposure in humans. Toxicol Appl Pharmacol 2005;206(2):113–20.

[14] Bradstreet J, Geier DA, Kartzinel JJ, Adams JB, Geier MR. A case-control study of mercury burden in children with autistic spectrum disorders. J Am Phys Surg 2003;8(3):76–9.

[15] Holmes AS, Blaxill MF, Haley BE. Reduced levels of mercury in first baby haircuts of autistic children. Int J Toxicol 2003;22(4):277-85.

[16] Heyer NJ, Echeverria D, Bittner AC Jr, Farin FM, Garabedian CC, Woods JS. Chronic low-level mercury exposure, BDNF polymorphism, and associations with self-reported symptoms and mood. Toxicol Sci 2004; 81(2):354–63.

[17] Hashimoto K, Iwata Y, Nakamura K, Tsujii M, Tsuchiya KJ, Sekine Y, Suzuki K, Minabe Y, Takei N, Iyo M, Mori N. Reduced serum levels of brain-derived neurotrophic factor in adult male patients with autism. Prog Neuropsychopharmacol Biol Psychiatry 2006;30(8):1529–31.

[18] Woods JS. Attenuation of porphyrinogen oxidation by glutathione in vitro and reversal by porphyrinogenic trace metals. Biochem Biophys Res Commun 1988;152(3):1428–34.

[19] James SJ, Cutler P, Melnyk S, Jernigan S, Janak L, Gaylor DW, Neubrander JA. Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism. Am J Clin Nutr 2004;80(6):1611–7.

[20] Nataf R, Skorupka C, Amet L, Lam A, Springbett A, Lathe R. Porphyrinuria in childhood autistic disorder: implications for environmental toxicity. Toxicol Appl Pharmacol 2006;214(2):99–108.

[21] Willmore LJ, Sypert GW, Munson JB. Recurrent seizures induced by cortical iron injection: A model of posttraumatic epilepsy. Ann Neurol 1978;4(4):329–36.

[22] Abbaslou P, Zaman T. A child with elemental mercury poisoning and unusual brain MRI findings. Clin Toxicol (Phila) 2006;44(1):85–8.

[23] Geier DA, Geier MR. A prospective assessment of porphyrins in autistic disorders: a potential marker for toxic metal exposure. Neurotox Res 2006;10(1):57–64.

[24] Ruscito BJ, Harrison NL. Hemoglobin metabolites mimic benzodiazepines and are possible mediators of hepatic encephalopathy. Blood 2003;102(4):1525–8.

[25] Riopelle RJ, Kennedy JC. Some aspects of porphyrin neurotoxicity in vitro. Can J Physiol Pharmacol 1982;60(5):707–14.

[26] Trasande L, Landrigan PJ, Schechter C. Public health and economic consequences of methyl mercury toxicity to the developing brain. Environ Health Perspect 2005;113(5):590–6.

[27] Bjornberg KA, Vahter M, Petersson-Grawe K, Glynn A, Cnattingius S, Darnerud PO, Atuma S, Aune M, Becker W, Berglund M. Methyl mercury and inorganic mercury in Swedish pregnant women and in cord blood: influence of fish consumption. Environ Health Perspect 2003; 111(4);637–41.

[28] Ramirez GB, Cruz CV, Pagulayan O, Ostrea E, Dalisay C. The Tagum study I: analysis and clinical correlates of mercury in maternal and cord blood, breast milk, meconium, and infant's hair. Pediatrics 2000;106:774–81.

[29] Sandborgh-Englund G, Ask K, Belfrage E, Ekstrand J. Mercury exposure in utero and during infancy. Journal of Toxicology and Environmental Health 2001;63:317–20.

[30] Gulson BL, Mizon KJ, Palmer JM, Korsch MJ, Taylor AJ, Mahaffey KR. Blood lead changes during pregnancy and postpartum with calcium supplementation. Environ Health Perspect 2004;112(15):1499–507.

[31] Martin MD, McCann T, Naleway C, Woods JS, Leroux BG, Bollen AM. The validity of spot urine samples for low-level occupational mercury exposure assessment and relationship to porphyrin and creatinine excretion rates. J Pharmacol Exp Ther 1996;277(1):239-44.

[32] Woods JS, Martin MD, Leroux BG. Validity of Spot Urine Samples as a Surrogate Measure of 24-Hour Porphyrin Excretion Rates: Evaluation of Diurnal Variations in Porphyrin, Mercury, and Creatinine Concentrations Among Subjects With Very Low Occupational Mercury Exposure. Journal of Occupational & Environmental Medicine 1998;40(12):1090–1101.

[33] Whiteley P, Waring R, Williams L, Klovrza L, Nolan F, Smith S, Farrow M, Dodou K, Lough WJ, Shattock P. Spot urinary creatinine excretion in pervasive developmental disorders. Pediatr Int 2006;48(3):292–7.

[34] D'Amelio M, Ricci I, Sacco R, et al. Paraoxonase gene variants are associated with autism in North America, but not in Italy: possible regional specificity in gene-environment interactions. Mol Psychiatry 2005;10(11):1006–16.

[35] Eppright TD, Sanfacon JA, Horwitz EA. Attention deficit hyperactivity disorder, infantile autism, and elevated blood-lead: a possible relationship. Missouri Medicine 1996;93(3):136–8.

[36] Waring RH, Klovrza LV. Sulphur metabolism in autism. Journal of Nutritional and Environmental Medicine 2000;10:25–32.

[37] Stangle DE, Strawderman MS, Smith D, Kuypers M, Strupp BJ. Reductions in blood lead overestimate reductions in brain lead following repeated succimer regimens in a rodent model of childhood lead exposure. Environ Health Perspect 2004;112(3):302–8.