A prospective study of prenatal mercury exposure from maternal dental amalgams and autism severity

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Dental amalgams containing 50% mercury (Hg) have been used in dentistry for the last 150 years, and Hg exposure during key developmental periods was associated with autism spectrum disorders (ASDs). This study examined increased Hg exposure from maternal dental amalgams during pregnancy among 100 qualifying participants born between 1990–1999 and diagnosed with DSM-IV autism (severe) or ASD (mild). Logistic regression analysis (age, gender, race, and region of residency adjusted) by quintile of maternal dental amalgams during pregnancy revealed the ratio of autism:ASD (severe:mild) were about 1 (no effect) for \(\leq5\) amalgams and increased for \(\geq6\) amalgams. Subjects with \(\geq6\) amalgams were 3.2-fold significantly more likely to be diagnosed with autism (severe), in comparison to ASD (mild), than subjects with \(\leq5\) amalgams. Dental amalgam policies should consider Hg exposure in women before and during the child-bearing age and the possibility of subsequent fetal exposure and adverse outcomes.

Key words: Asperger’s syndrome, autism, developmental delay, neurodevelopmental disorder

INTRODUCTION

The practice of using amalgams (which generally contain 50% mercury) in dentistry has existed for over 150 years. As of mid-2008, the US Food and Drug Administration (FDA) has declined to classify the medical-device safety of amalgams used in dentistry. The American Dental Association maintains that the mercury in amalgam is safe and that the mercury does not leak (Edlich et al. 2007).

Yet, the research evidence suggests that there is significant amount of elemental leaching and mercury vapor release from amalgams (Cohen and Penugonda 2001) and that this liberated mercury is absorbed by several body tissues (Mutter et al. 2004, Edlich et al. 2007). As a result, dental amalgams are a significant source of mercury body burden, as studies in animals and humans show (Mutter et al. 2007). For example, Guzzi and coworkers (2006) found that, on autopsy, total mercury levels were significantly higher in subjects with a greater number of amalgam surfaces (\(>12\)) compared with those who had fewer (0–3), in all types of tissue. These authors also reported that the greater the number of amalgams, the greater the likelihood that mercury would be found in the brain. In regard to amalgam bearers, other investigators have reported an approximate 2- to 5-fold increase of the mercury level in blood and urine as well as a 2- to 12-fold increase of the mercury concentration in several body tissues (Mutter et al. 2007). Also, mercury from maternal amalgam fillings leads to a significant increase of mercury concentration in the tissues and the hair of fetuses and newborn children. Furthermore, placental, fetal, and infant mercury body burden correlates with the numbers of amalgam fillings of the mothers (Mutter et al. 2007, Palkovicova et al. 2008). Finally, mercury levels in amniotic fluid and breast milk correlate significantly with the number of maternal dental amalgam fillings (Mutter et al. 2007).

The overall importance of dental amalgams, particularly maternal dental amalgams, significantly contributing to fetal and early infant mercury body-burden stems from the fact that recent studies have postulated that mercury exposure can cause immune, sensory, neu-
logical, motor, and behavioral dysfunctions similar to traits defining or associated with autism spectrum disorders (ASDs), and that these similarities extend to neuroanatomy, neurotransmitters, and biochemistry (Mutter et al. 2005, Kern and Jones 2006, Maya and Luna 2006, Austin 2008, Geier et al. 2008b). In addition, investigators from the US National Institute of Environmental Health Sciences (1999) and the National Institute for Occupation Safety and Health of the Centers for Disease Control and Prevention (Nelson 1991) have described a role for mercury exposure in the pathogenesis of autism. Mercury poisoning has also sometimes been presumptively diagnosed as autism of unknown etiology until mercury poisoning has been established (Chrysochoou et al. 2003) and other investigators have reported on a case-series of patients diagnosed with mercury-induced ASDs (Geier and Geier 2007a). Further, Faustman and others (2000) reporting on the effects of mercury on neuronal development stated: “(...) mercury exposure altered cell number and cell division; these impacts have been postulated as modes of action for the observed adverse effects in neuronal development. The potential implications of such observations are evident when evaluated in context with research showing that altered cell proliferation and focal neuropathologic effects have been linked with specific neurobehavioral deficits (e.g., autism).” Finally, the Collaborative on Health and the Environment’s Learning and Developmental Disabilities (2008) recently published a consensus statement reporting that there is no doubt mercury exposure may produce ASDs.

Based upon the foregoing, it was hypothesized that mercury exposure from maternal dental amalgams during pregnancy may significantly impact the severity of ASD diagnoses. In order to evaluate this hypothesis, the present prospective, blinded study was designed to examine the relationship between mercury exposure from maternal dental amalgams during pregnancy and the severity of subjects diagnosed with an ASD. Further, the purpose of the analysis was to determine if there were a threshold number of maternal dental amalgams during pregnancy above which there was an increase in the severity of subjects diagnosed with an ASD.

METHODS

The study protocol received Institutional Review Board (IRB) approval from the Institute of Chronic Illnesses, Inc. (Silver Spring, MD).

Participants

The present study looked at 100 qualifying participants who were prospectively recruited from patients presenting for outpatient genetic consultations at the Genetic Centers of America. All of the children were previously diagnosed with Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM–IV) criteria autism or pervasive developmental delay (PDD) by a trained professional. Children included in the present study had Rh-positive mothers, were at least 6 years-old at the time of initial clinical presentation, and were born between 1990 through 1999. Children were excluded from the present study with known prenatal exposure to mercury-containing drugs (i.e. Rho(D) immune globulins, influenza vaccinations, etc.).

Clinical Evaluation

At the time of their initial clinical presentation, the children examined in the present study had extensive medical histories taken. Information collected on the subjects examined included: race, gender, region of residency, and number of maternal amalgams present during pregnancy. In addition, a parent/guardian of each child participated in a psychiatric interview about their son/daughter and each child was examined to evaluate autistic symptom severity by clinical observation and Autism Treatment Evaluation Checklist (ATEC) (Autism Research Institute, San Diego, CA) scoring. The ATEC evaluates skills in a number of areas, including speech language/communication, sociability, sensory/cognitive awareness, and health/physical/behavior. Based upon this information, a trained physician, using the International Classification of Disease, 9th Revision, diagnosed each patient with autism (299.00, severe clinical presentation) or an ASD (299.80, mild clinical presentation).

Statistical Evaluation

In order to determine the relationship between number of maternal dental amalgams during pregnancy and the risk of diagnosed autism (severe) in comparison to diagnosed ASD (mild) and, specifically, to determine if there were a threshold number of maternal dental amalgams during pregnancy above which there is an increased risk of diagnosed autism (severe)
Table I

A summary of the participants with autism spectrum disorders examined

<table>
<thead>
<tr>
<th>Descriptive Information</th>
<th>Overall ((n=100))</th>
<th>ASD (mild)(^1) ((n=60))</th>
<th>Autism (severe)(^2) ((n=40))</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male / Female (ratio)</td>
<td>85/15 ((5.7:1))</td>
<td>51/9 ((5.7:1))</td>
<td>34/6 ((5.7:1))</td>
<td>–</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td>0.54</td>
</tr>
<tr>
<td>Mean Age in Years ± SD</td>
<td>10.4 ± 2.6</td>
<td>10.5 ± 2.5</td>
<td>10.2 ± 2.8</td>
<td></td>
</tr>
<tr>
<td>Race ((n))</td>
<td></td>
<td></td>
<td></td>
<td>0.12</td>
</tr>
<tr>
<td>Caucasian</td>
<td>77% ((77))</td>
<td>72% ((43))</td>
<td>85% ((34))</td>
<td></td>
</tr>
<tr>
<td>Minorities(^3)</td>
<td>23% ((23))</td>
<td>28% ((17))</td>
<td>15% ((6))</td>
<td></td>
</tr>
<tr>
<td>Region of Residency ((n))</td>
<td></td>
<td></td>
<td></td>
<td>0.48</td>
</tr>
<tr>
<td>Mid-west</td>
<td>19% ((19))</td>
<td>16.7% ((10))</td>
<td>22.5% ((9))</td>
<td></td>
</tr>
<tr>
<td>Northeast</td>
<td>12% ((12))</td>
<td>8.3% ((5))</td>
<td>17.5% ((7))</td>
<td></td>
</tr>
<tr>
<td>Southeast</td>
<td>55% ((55))</td>
<td>61.7% ((37))</td>
<td>45% ((18))</td>
<td></td>
</tr>
<tr>
<td>Southwest</td>
<td>3% ((3))</td>
<td>3.3% ((2))</td>
<td>2.5% ((1))</td>
<td></td>
</tr>
<tr>
<td>West</td>
<td>11% ((11))</td>
<td>10.0% ((6))</td>
<td>12.5% ((5))</td>
<td></td>
</tr>
<tr>
<td>Mean Maternal Dental Amalgams During Pregnancy ± SD</td>
<td></td>
<td></td>
<td></td>
<td>0.095(^4)</td>
</tr>
<tr>
<td></td>
<td>4.4 ± 4.2</td>
<td>3.6 ± 3.0</td>
<td>5.5 ± 6.0</td>
<td></td>
</tr>
</tbody>
</table>

(ASD) autism spectrum disorder; (SD) standard deviation. All participants examined in the present study had Rh-positive mothers, were born between 1990 through 1999, and had no known significant prenatal exposure to mercury-containing drugs (i.e. Rho(D) immune globulins, influenza vaccinations, etc.). Mid-west (states: CO, IL, KS, MN, MO, UT), northeast (states: CT, MA, NH, NJ, NY, PA), southeast (states: DC, FL, GA, KY, MD, NC, SC, TN, VA), southwest (states: AZ, TX), and west (states: CA, HI, WA). (1) ASD is defined as any study participant diagnosed with autism spectrum disorder \((299.80, \text{mild clinical presentation})\) by a trained physician using the International Classification of Disease, 9\(^{th}\) revision criteria following a psychiatric interview. (2) Autism is defined as any study participant diagnosed with autism \((299.00, \text{severe clinical presentation})\) by a trained physician using the International Classification of Disease, 9\(^{th}\) revision criteria following a psychiatric interview. (3) Includes participants of Black, Indian, Mixed, or Oriental Ancestry. (4) After adjusting for age, gender, race, and region the mean number of amalgams was 4.6 for those with an autism diagnosis and 3.1 for those with an ASD diagnosis, but the difference did not reach statistical significance.
in comparison to diagnosed ASD (mild), a logistic regression analysis was used. To assess the form of this relationship, the data were divided into quintiles, denoting the number of maternal dental amalgams during pregnancy and the odds of diagnosed autism (severe) in comparison to diagnosed ASD (mild) among the highest exposed subjects, relative to the lowest exposed subjects. The data entered into the logistic regression model was adjusted for age, sex, race, and region of residency. The null hypothesis was that the number of maternal dental amalgams during pregnancy would have no effect on autism severity. A two-sided \( P \)-value <0.05 was considered statistically significant.

**RESULTS**

The dataset contained data on 100 subjects, 40 subjects diagnosed with autism and 60 subjects with diagnosed with an ASD. Table I summarizes the overall demographic data collected on the subjects examined in the present study. There were no statistically significant differences in gender, age, race, or region of residency among the subjects examined.

The mean number of maternal dental amalgams during pregnancy was 4.4 ± 4.2 with numbers ranging from 0 to 15. Among males (\( n=85 \)) the mean number of maternal dental amalgams during pregnancy was 4.4 ± 4.4 and among females (\( n=15 \)) was 4.7 ± 3.1. Among whites (\( n=77 \)) the mean was 5.0 ± 4.4 while for nonwhites (\( n=23 \)) it was 2.3 ± 2.6. The mean for the mid-west was 4.8 ± 4.0, northeast was 4.0 ± 3.0, southeast was 4.1 ± 4.8, southwest was 5.7 ± 2.1, and west was 5.1 ± 3.5.

For those with an autism (severe) diagnosis, the mean number of maternal dental amalgams during pregnancy was 5.5 ± 6.0 while for those with an ASD (mild) diagnosis the mean was 3.6 ± 3.0. After adjusting for age, gender, race, and region, the mean number of amalgams was 4.6 for those with a diagnosis of autism (severe) and 3.1 for those with an ASD (mild) diagnosis, but the difference did not reach statistical significance (\( \chi^2=2.8, \text{ df}=1, P=0.0946 \)).

Table II examines the relationship between number of maternal dental amalgams during pregnancy and the risk of subject being diagnosed with autism (severe) in comparison to an ASD (mild). Specifically, Table II examines whether there were a threshold number of maternal dental amalgams during pregnancy above which there is an increased risk of a diagnosis of autism (severe), in comparison to a diagnosis of an ASD (mild). Logistic regression analysis was used to assess the odds of a subject being diagnosed with autism (severe) in comparison to an ASD (mild), relative to lowest level of exposure to maternal dental amalgams during pregnancy, for each quintile of maternal dental amalgams during pregnancy examined. The odds of autism (severe) were about 1 (no effect) for 5 or fewer maternal dental amalgams during pregnancy and increased for 6 or more amalgams, although the odds ratio was only significantly greater than 1 for the highest quintile (8 or more amalgams). This result suggests that the odds of a subject being diagnosed with autism (severe) in comparison to an ASD (mild) only increased with 6 or more maternal amalgams.

For those with an autism (severe) diagnosis, the mean number of maternal dental amalgams during pregnancy was 5.5 ± 6.0 while for those with an ASD (mild) diagnosis the mean was 3.6 ± 3.0. After adjusting for age, gender, race, and region, the mean number of amalgams was 4.6 for those with a diagnosis of autism (severe) and 3.1 for those with an ASD (mild) diagnosis, but the difference did not reach statistical significance (\( \chi^2=2.8, \text{ df}=1, P=0.0946 \)).

**DISCUSSION**

The present study is the first prospective, blinded study to examine the relationship between mercury exposure from maternal dental amalgams and its association with severity of ASDs diagnoses. Among the study subjects, the highest exposures to mercury from maternal dental amalgams during pregnancy were associated with an increased risk of being diagnosed with autism (severe clinical symptoms), in comparison to an ASD (mild clinical symptoms). Furthermore, the risk of increasing autism severity became significantly manifest among those study subjects with 6 or more maternal dental amalgams during pregnancy in comparison to those study subjects with 5 or fewer maternal amalgams (\( \chi^2=6.2, \text{ df}=1, P=0.0127 \)). This model was adjusted for age, gender, race, and region of residency.
In considering the results of the present study in the context of previous studies, Holmes and coauthors (2003) examined prenatal sources of mercury exposure among patients diagnosed with autism in comparison to matched controls. It was observed that patients diagnosed with autism (8.35 ± 3.43) had exposure from significantly increased numbers of maternal dental amalgams during pregnancy than controls (6.60 ± 3.55). In contrast, Adams and others (2008) observed that patients diagnosed with autism (5.5 ± 4.2) had similar numbers of maternal dental amalgams during pregnancy as matched controls (6.6 ± 3.6).

Among the problems with the conflicting observations made in the aforementioned studies is that there may be potential social or medical biases associated with collection of cases or controls that influence the data collected regarding the number of maternal dental amalgams during pregnancy. This type of effect has been described in previous studies, and if not properly taken into account, it may bias statistical measures towards the null hypothesis (Fine and Chen 1992).

The advantage of the present study over the previous studies is that the present study was designed to examine a prospective sample of patients all diagnosed previously on the autism spectrum. As a result, potential influences associated with collection of cases or controls that may have adversely impacted previous sample collections, such as motivating factors to present to an autism clinic for therapy or a desire for a control to participate in a study, would not adversely affect the present sample, since every patient diagnosed with an ASD examined in the present study presented on a prospective basis to a single autism clinic.

It is also interesting to note that the overall mean for maternal dental amalgams observed among subjects diagnosed with autism in the present study at 5.5 is the overall mean for maternal dental amalgams observed among subjects diagnosed with autism in the present study at 5.5 is the

<table>
<thead>
<tr>
<th>Maternal Dental Amalgams During Pregnancy (n)</th>
<th>Odds of Autism Diagnosis (severe) vs. ASD Diagnosis (mild)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (29)</td>
<td>1.0</td>
<td>(reference group)</td>
</tr>
<tr>
<td>1 to 2 (11)</td>
<td>1.3</td>
<td>0.7325</td>
</tr>
<tr>
<td>3 to 5 [25]</td>
<td>1.3</td>
<td>0.6686</td>
</tr>
<tr>
<td>6 to 7 (17)</td>
<td>3.3</td>
<td>0.0932</td>
</tr>
<tr>
<td>8+ (18)</td>
<td>4.4</td>
<td>0.0333</td>
</tr>
<tr>
<td>≤ 5 (65)</td>
<td>1.0</td>
<td>(reference group)</td>
</tr>
<tr>
<td>≥ 6 (35)</td>
<td>3.2</td>
<td>0.0127</td>
</tr>
</tbody>
</table>

(ASD) autism spectrum disorder. (1) These statistical tests were adjusted for age, gender, race, and region of residency of the study subjects.
same as was observed by Adams and coworkers (2008), and helps to further indicate that potential biases associated with autism clinics may have limited impact on the present study. Additionally, an examination of the raw data from the Adams and others (2008) study (provided by these researchers to the present investigators) revealed that, among subjects diagnosed with autism whose mothers were not administered Rho(D) immune globulins, there was a significant increase in mean maternal dental amalgams during pregnancy ($6.7 \pm 3.8$ vs. $3.9 \pm 3.8$, $t=-2.8$, df=57, $P<0.01$), when comparing those with severe autistic symptoms (score $>$ sample median) in comparison to those with mild autistic symptoms (score $<$ sample median). This finding is consistent with the observations made in the present study.

The design of the present study also has advantages over those of previous studies because specific measures were employed to control for sources of prenatal mercury exposure other than dental amalgams that might confound the results. These measures consisted of excluding Rh-negative mothers (i.e. to eliminate potential mercury exposure from Rho(D) immune globulins administered during pregnancy) from the study as well as mothers who had other identifiable sources of medicinal mercury exposure (i.e. Thimerosal-containing vaccine administration).

The analysis method employed in the present study is significantly different from several recent clinical trials that have evaluated dental amalgam safety in children and deemed the potential neurobehavioral or neurological effects from dental amalgam mercury exposure in children to be inconsequential (Lauterbach et al. 2008). For example, these trials examined the number of amalgams in the children ranging in age from 6–12 years of age (Bellinger et al. 2006, DeRouen et al. 2006, Lauterbach et al. 2008). The present study is different in that it examines maternal dental amalgams and fetal exposure, and as a result, represents a narrow window of exposure that is much earlier.

Age, or developmental period, at the time of exposure is clearly an important factor in determining the impact of toxic exposures. The impact of toxic compounds in the body is a function of developmental age (Makri et al. 2004). Infant and fetal tissue is less resistant to toxic effects than that of older children and adults (Graeter and Mortensen 1996). In rats, for example, the main route of elimination of methylmercury is by secreting the toxin into bile. In neonatal rats, this ability to secrete mercury into bile develops between 2 and 4 weeks of age and is correlated with the increasing ability of the developing liver to secrete glutathione into bile. Prior to 2–4 weeks of age, neonatal rats are more vulnerable to mercury toxicity (Ballatori and Clarkson 1982).

As was stated previously, evidence also shows that mercury from the mother reaches the fetus. Palkovicova and colleagues (2008), for example, found a strong positive correlation between human maternal and cord blood mercury levels. Levels of mercury in the cord blood were significantly associated with the number of maternal amalgam fillings and with the number of years since the last filling. In addition, Drasch and others (1994) found that blood mercury levels in one-day old human infants and older infants correlated significantly with the number of dental amalgam fillings of the mother. Clearly, the fetus is particularly vulnerable to the toxic effects of maternal sources of mercury in utero, during a critical period of neurological development, when the body is least adept at excreting the toxin.

The results observed in the present study associate the severity of autism diagnosed in a child with exposure to mercury from maternal dental amalgams, a finding which is consistent with a previously described case-series study. The case-series study revealed a significant association between total mercury exposure during the prenatal and early postnatal periods from Thimerosal-containing vaccines/biologics and the severity of autism, as measured using an ATEC form (Geier and Geier 2007a). Providing further corroboration still are population epidemiologic studies which have found a significant association between increasing mercury exposure from vapor (Palmer et al. 2006, 2009) or administration of Thimerosal-containing biologics/vaccines (Geier et al. 2008a, Young et al. 2008) and the risk for individuals being diagnosed with an ASD.

porphyrins associated with mercury body-burden/toxicity, that these biomarkers were found to significantly increase across the autism spectrum (autism > PDD > Asperger’s disorder) (Geier and Geier 2006, 2007b, Nataf et al. 2006, 2008 Austin and Shandley 2008). Furthermore, a recent study also demonstrated a significant association between increasing autism severity, as measured by the Childhood Autism Rating Scale (CARS), and increasing urinary porphyrins associated with mercury body-burden/toxicity (Geier et al. 2009). Finally, it was even recently reported that levels of glutathione, a substance produced by the body that is very important to heavy metal detoxification, were found to significantly decrease across the autism spectrum (Asperger’s disorder > PDD > autism). These investigators reported that their observations suggest that glutathione levels play an important functional role in helping to dictate autism severity following mercury intoxication (Pasca et al. 2008).

Limitations of the present study
In considering the limitations of the present study, it was not possible to examine potential sources of mercury exposure from maternal fish consumption during pregnancy or from region-specific mercury exposure due to environmental sources. In order to help adjust for these potential confounders in the data, the data analyses conducted were adjusted for region of residency. In addition, it was not possible in the present study to examine specific early postnatal exposures to mercury from Thimerosal-containing vaccines. In order to help adjust for this potential confounder in the data, the children included in this study were born from 1990 through 1999; this served to minimize potential differences in mercury exposure from Thimerosal-containing vaccines as result of different childhood vaccine schedules pre-1990 and post-1999. Since these potential confounders could not be fully accounted for, children examined in the present study may have been misclassified with regards to their total cumulative exposure to mercury during fetal and early infant periods. The overall effect is that these confounders may have introduced statistical noise, biasing the statistical measures toward to the null hypothesis and helping to minimize the magnitude of the observed effects. It would be worthwhile in future studies to attempt to examine these other sources of mercury during fetal and early infant periods and to consider how they might interact with mercury exposure from maternal dental amalgams during pregnancy.

In addition, since information on the numbers of fillings but not information on: their size (surface area), the nature of the amalgam alloy used, or the location of the amalgam in the mouth was available to researchers in this study, the effects of: the amalgam size (surface area) of the fillings; the intrinsic mercury release rates for the amalgam composition installed; and chewing friction, are confounders that further reduce the magnitude of the observed effects. Future studies should examine these parameters and how they relate to cumulative mercury exposure.

Another limitation of the present study was that ASD severity was assessed based upon diagnosis (i.e. autism vs. ASD) and was not based upon a continuous variable of ASD severity. The present study, a prospective, blinded study, did have the advantage that potential medical evaluation biases that might influence diagnosis severity or estimates of maternal dental amalgams during pregnancy were minimized because these two measurements were made blinded to one another. It would be worthwhile in futures studies to attempt to use other measures of autism severity such as autism severity, as measured by CARS scores, and its relationship to mercury exposure, as measured by both amalgam number and estimated amalgam surface area.

CONCLUSIONS
The present study is the first prospective, blinded epidemiological study to evaluate the relationship between mercury exposure from maternal dental amalgams during pregnancy and its relationship with the severity of diagnosed ASDs. This study helps to demonstrate that elevated mercury exposure from maternal dental amalgams during pregnancy is associated with an elevated risk of being diagnosed with autism (severe clinical symptoms), in comparison to an ASD (mild clinical symptoms), and that the risk of increasing autism severity became apparent at the threshold of 6 or more maternal dental amalgams during pregnancy. The observations made in the present study are consistent with recently emerging evidence showing that there is a significant relationship between mercury exposure, particularly in the fetal and early infant temporal periods, and the subsequent risk of patients being diagnosed on the autism spectrum. Evidence from the present study, combined with other published research, suggests that policies on the use of
dental amalgams should carefully consider the issue of mercury exposure in women before and during the child-bearing age and the possibility of subsequent fetal exposure and adverse outcomes. Future studies should be conducted to further evaluate the critical relationship between mercury exposure from dental amalgams during fetal and early infant temporal periods and the subsequent risk of these children developing neurodevelopmental disorders.

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