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ORIGINAL ARTICLE

Thimerosal exposure and disturbance of emotions specific to childhood and adolescence: A case-control study in the Vaccine Safety Datalink (VSD) database

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ABSTRACT

Background: This study evaluated Thimerosal-containing childhood vaccines and the risk of a diagnosis called disturbance of emotions specific to childhood and adolescence (ED). Thimerosal is an organic-mercury (Hg)-containing compound used in some vaccines.

Methods: A hypothesis-testing prospective, longitudinal case-control study evaluated Hg exposure from Thimerosal in hepatitis B vaccines administered at specific times within the first 6 months of life and its association with medically diagnosed ED (313.xx) (n = 517) in children born between 1991–2000 in comparison to controls (n = 27 491) in the Vaccine Safety Datalink (VSD) database.

Results: Cases diagnosed with ED were significantly more likely than controls to have received increased Hg exposure within the first month of life (odds ratio (OR) = 1.3384), the first 2 months of life (OR = 1.3367) and the first 6 months of life (OR = 2.37). When the data were separated by gender, similar significant adverse effects were observed for males, but not females. On a per microgram Hg basis, cases diagnosed with ED were significantly more likely than controls to have received increased exposure within the first 6 months of life (OR = 1.025 per microgram Hg).

Conclusions: The results show a significant relationship between Hg exposure from Thimerosal-containing childhood vaccines and the subsequent risk of an ED diagnosis.

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Introduction

The International Classification of Diseases, Ninth Revision (ICD-9) code 313.xx is entitled, 'Disturbance of emotions specific to childhood and adolescence' (emotional disturbances (ED)). The following comprise the different types of ED diagnoses: (1) over-anxious disorder specific to childhood and adolescence; (2) misery and unhappiness disorder specific to childhood and adolescence; (3) shyness disorder of childhood; (4) introverted disorder of childhood; (5) selective mutism; (6) relationship problems specific to childhood and adolescence; (7) oppositional defiance disorder; (8) identity disorder of childhood or adolescence; (9) academic underachievement disorder of childhood or adolescence; (10) other emotional disturbances of childhood or adolescence; and (11) unspecified emotional disturbance of childhood or adolescence [1].

According to a 2005 analysis, as of 2004, there were ~ 450 000 students diagnosed with an ED in the US population, ~ 9% of all students [2]. They also reported that over 75% of youth classified as having an ED diagnosis were boys, and there was a wide range of co-morbid diagnoses, including anxiety, bipolar disorder, depression, oppositional behaviour, psychosis, attention deficit hyperactivity disorder (ADHD) and learning disability (LD). Approximately two-thirds had a co-morbid diagnosis of ADHD and approximately one-fourth had a co-morbid diagnosis of LD. In addition, children diagnosed with an ED have poorer societal outcomes than the general population and have high rates of criminal justice involvement [3].

At the present time there is no consensus on the cause of ED. Investigators previously described that interacting genetic, environmental and social factors are important determinants. Mercury (Hg) is a neurodevelopmental toxicant and extensive laboratory and clinical studies of Hg demonstrate the unique vulnerability of the developing brain to Hg [4].

In considering the sources of Hg exposure to infants, Thimerosal (sodium ethyl-Hg thiosalicylate, $C_9H_9HgNaO_2S$) is an ethylmercury-containing compound (49.55% Hg by weight) that was and continues to be utilized in vaccines that are routinely administered to pregnant women and infants in the US and worldwide [5].

In the US, prenatal exposure to Hg results from the routine recommendation to administer influenza vaccines to pregnant women at any time during pregnancy [5]. In addition, postnatal exposure to Hg results from the routine recommendation to administer three doses of influenza vaccine during the first 18 months of life, and then throughout childhood on an annual basis [5]. It is estimated that about half of the doses of influenza vaccine in the US still contain Thimerosal [5]. Worldwide, and in particular in developing nations, Thimerosal is still present in many vaccines routinely administered to infants/children [5]. It was previously estimated that ~ 50% of the Hg dose that some infants receive is from Thimerosal-

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containing vaccines administered within the first 6 months of life, the balance being derived from breast milk [6].

A previous review of the literature extensively examined biological mechanisms and susceptibility to Thimerosal toxicity [5]. It was described that Thimerosal, when dissolved in aqueous solutions, rapidly disassociates into ethy-Hg hydroxide (ethyl-Hg-OH), ethylmercury chloride (ethyl-Hg-Cl), and thiosalcylate. It was reported that administration of Thimerosal according to the childhood vaccine schedule rapidly resulted in significantly increased brain Hg levels in the forms of ethyl-Hg, methyl-Hg, and inorganic Hg. It was also reported that significant amounts of Hg remained in the brain long after there were no detectable Hg levels in the blood. In further examining the persistence of Hg in the brain, inorganic Hg appeared to remain in the brain for the longest period of time, and was estimated to remain in the brain for many years or even decades following exposure. It was delineated that Thimerosal neurotoxicity was associated with oxidative stress, brain inflammation, auto-antibody formation against brain tissue, impairment in methylation, decreased neuronal cell count, mitochondrial dysfunction, and decreased cerebral/cerebellar flood flow. Finally, it was revealed that there is a portion of the human population that is biochemically susceptible to Thimerosal neurotoxicity [5].

Unfortunately, the recommendations for the routine administration of Thimerosal-containing vaccines to pregnant women and children never considered that a portion of the human population might be biochemically susceptible to Thimerosal neurotoxicity. As a consequence, the purpose of this study was to examine prospectively collected medical records as part of the routine clinical care provided to infants and children and evaluate the potential relationship between exposure to Hg from Thimerosal-containing infant vaccines and the long-term risk of a child being diagnosed with an ED.

Materials and methods

Institutional review board approval

The study protocol was approved by the US Centers for Disease Control and Prevention (CDC), the Institutional Review Board (IRB) of Kaiser Permanente North-West (KPNW), and the IRB of Kaiser Permanente Northern California (KPNC). The data were analyzed at the secure Research Data Center of the National Center for Health Statistics in Hyattsville, MD. The views expressed in this study do not necessarily reflect those of the CDC or those of Kaiser Permanente.

The Vaccine Safety Datalink (VSD) project was created in 1991 by the National Immunization Program (NIP) of the CDC. The VSD's data collection and study methods were previously described [7–9]. The VSD project links medical event information, specific vaccine history, and selected demographic information from the computerized databases of several health maintenance organizations (HMOs).

Determining the population at risk

A cohort of over 1 million infants enrolled in the VSD project (the CDC provided data to independent investigators that was updated through the end of 2000) from KPNW, Kaiser Permanente Colorado (KPC) and KPNC was examined using SAS® software. The cohort examined was restricted to the accessible records for individuals who were continuously HMO-enrolled from their date of birth and whose records specified their gender.

Determining cases

The outcome files (inpatient and outpatient diagnoses) from this population were then reviewed to find the first instance of an ED diagnosis for each child, as defined by the International Classification of Disease, 9th revision (ICD-9) code 313.xx. The codes examined included: (1) overanxious disorder specific to childhood and adolescence (313.0); (2) misery and unhappiness disorder specific to childhood and adolescence (313.1); (3) shyness disorder of childhood (313.21); (4) introverted disorder of childhood (313.22); (5) selective mutism (313.23); (6) relationship problems specific to childhood and adolescence (313.3); (7) oppositional defiance disorder (313.81); (8) identity disorder of childhood or adolescence (313.82); (9) academic under-achievement disorder of childhood or adolescence (313.83); (10) other emotional disturbances of childhood or adolescence (313.89); and (11) unspecified emotional disturbance of childhood or adolescence (313.9).

When there were multiple instances of the same diagnosis in a child, only the first instance was counted. In addition, to ensure the potential for an association between exposure and outcome, for vaccinated individuals diagnosed with ED, only those individuals diagnosed with ED following the vaccines under study were included as cases in the present analyses. Applying this criterion, the study identified 517 cases diagnosed with ED (males = 399, females = 118, male/female ratio = 3.38) born from 1991–2000.

Determining controls

Working within the restrictions of the available data, controls without a diagnosis of ED who would have only a minimal chance of subsequently receiving such a diagnosis (i.e. to minimize the risk of misclassification of controls) were identified. The selection criterion for these controls was that they had to be continuously enrolled from birth for at least 7.13 years (mean age of initial diagnosis of ED plus the standard deviation of mean age of initial diagnosis of ED). Applying this criterion, the study identified 27 491 controls without an ED diagnosis (males = 14 013, females = 13 478, male/female ratio = 1.04) born from 1991–1993.

Statistical analysis

In all statistical analyses, a two-sided probability value (p-value) of < 0.05 was considered statistically significant and odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. The null hypothesis for each statistical test was that there would be no difference in exposure to Thimerosal-containing hepatitis B vaccine among cases diagnosed with an ED in comparison to controls.

In the first set of comparisons (Exposure Groups I–III), exposure to Hg from Thimerosal-containing hepatitis B vaccines administered within the first month of life (12.5 μg Hg vs 0 μg Hg), within the first 2 months of life (25 μg Hg vs 0 μg Hg) and within the first 6 months of life (37.5 μg Hg vs 0 μg Hg) were compared among cases diagnosed with an ED in comparison to controls. In the next set of comparisons, the data were then separated by gender (males, Exposure Groups IV–VI; and females, Exposure Groups VII–IX) and similar analyses were undertaken to compare exposure among cases diagnosed with an ED in comparison to controls. All of these statistical analyses utilized the Fisher's exact test statistic in SAS software. In the final set of comparisons, continuous exposure to Hg from Thimerosal-containing hepatitis B vaccines administered within the first 6 months of life (0 μg Hg, 12.5 μg Hg, 25 μg Hg or 37.5 μg Hg) was evaluated among cases diagnosed with an ED in comparison to controls. This analysis utilized the logistic regression test statistic in StatsDirect software.

Results

Table 1 reveals exposure to Hg from Thimerosal-containing hepatitis B vaccine administration within the first 6 months of life among cases diagnosed with an ED in comparison to controls. It was observed that cases diagnosed with an ED in comparison to controls were significantly more likely to have received increased Hg exposure from Thimerosal-containing hepatitis B vaccines administered within the first month of life, within the first 2 months of life and within the first 6 months of life. The smallest effect was observed for cases diagnosed with an ED in comparison to controls for exposure group II (OR = 1.3367, 95% CI = 1.1178–1.5984) and the largest for exposure group III (OR = 2.37, 95% CI = 1.3016–4.3288).

Tables 2 and 3 evaluate exposure to Hg from Thimerosal-containing hepatitis B vaccines administered within the first 6 months of life among male cases compared to male controls and female cases compared to female controls, respectively. It was observed that male cases diagnosed with an ED in comparison to male controls were significantly more likely to have received increased Hg exposure from Thimerosal-containing hepatitis B vaccines administered within the first month of life, the first 2 months of life and the first 6 months of life. The smallest effect was observed for male cases diagnosed with an ED in comparison to male controls for exposure group V (OR = 1.3535, 95% CI = 1.1033–1.6604) and the largest for exposure group VI (OR = 2.2524, 95% CI = 1.1703–4.3350). In contrast, while there were increased ORs for increased exposure to Hg from Thimerosal-containing hepatitis B vaccines administered in each of the exposure periods examined for female cases diagnosed with an ED in comparison to female controls, there were no significant differences in exposure at any exposure time period examined.

Table 4 presents the results of logistic regression analysis of the effect of Hg exposure from Thimerosal-containing hepatitis B vaccines administered within the first 6 months of life among cases diagnosed with an ED in comparison to controls. It was observed that there was a significantly increased dose-response effect (OR = 1.025 per μg Hg, 95% CI = 1.008–1.043).

Table 1. A summary of exposure to Hg from Thimerosal-containing hepatitis B vaccine administration among cases diagnosed with emotional disturbances in comparison to controls[a] within the VSD database.

Group examined	Number of cases diagnosed with emotional disturbances (%)	Number of controls without an emotional disturbances diagnosis (%)	Odds ratio (95% CI)	*p*-value
Exposure Group I			1.3384 (1.12–1.5994)	< 0.005
12.5 μg Hg within 1st month	204 (39.46)	9003 (32.75)		
0 μg Hg within 1st month	313 (60.54)	18488 (67.25)		
Exposure Group II			1.3367 (1.1178–1.5984)	< 0.005
25 μg Hg within first 2 months	205 (40.20)	9017 (33.46)		
0 μg Hg within first 2 months	305 (59.80)	17932 (66.54)		
Exposure Group III			2.37 (1.3016–4.3288)	< 0.005
37.5 μg Hg within first 6 months	39 (72.22)	1252 (52.28)		
0 μg Hg within first 6 months	15 (27.78)	1143 (47.72)		

[a] These controls had to be continuously enrolled from birth for at least 7.13 years (mean age of initial diagnosis of emotional disturbances plus the standard deviation of mean age of initial diagnosis of emotional disturbances).
Hg, mercury; μg, microgram.

Table 2. A summary of exposure to Hg from Thimerosal-containing hepatitis B vaccine administration among male cases diagnosed with emotional disturbances in comparison to male controls[a] within the VSD database.

Group examined	Number of male cases diagnosed with emotional disturbances (%)	Number of male controls without an emotional disturbances diagnosis (%)	Odds ratio (95% CI)	*p*-value
Exposure Group IV			1.3556 (1.1057–1.6618)	< 0.005
12.5 μg Hg within 1st month	158 (39.60)	4568 (32.60)		
0 μg Hg within 1st month	241 (60.40)	9445 (67.40)		
Exposure Group V			1.3535 (1.1033–1.6604)	< 0.005
25 μg Hg within first 2 months	159 (40.36)	4575 (33.33)		
0 μg Hg within first 2 months	235 (59.64)	9152 (66.67)		
Exposure Group VI			2.2524 (1.1703–4.3350)	< 0.05
37.5 μg Hg within first 6 months	32 (71.11)	612 (52.22)		
0 μg Hg within first 6 months	13 (28.89)	560 (47.78)		

[a] These controls had to be continuously enrolled from birth for at least 7.13 years (mean age of initial diagnosis of emotional disturbances plus the standard deviation of mean age of initial diagnosis of emotional disturbances).
Hg, mercury; μg, microgram.

Table 3. A summary of exposure to Hg from Thimerosal-containing hepatitis B vaccine administration among female cases diagnosed with emotional disturbances in comparison to female controls[a] within the VSD database.

Group examined	Number of female cases diagnosed with emotional disturbances (%)	Number of female controls without an emotional disturbances diagnosis (%)	Odds ratio (95% CI)	*p*-value
Exposure Group VII			1.3027 (0.8983–1.8892)	> 0.15
12.5 μg Hg within 1st month	46 (38.98)	4435 (32.91)		
0 μg Hg within 1st month	72 (61.02)	9043 (67.09)		
Exposure Group VIII			1.2989 (0.8938–1.8875)	> 0.15
25 μg Hg within first 2 months	46 (39.66)	4442 (33.60)		
0 μg Hg within first 2 months	70 (60.34)	8780 (66.40)		
Exposure Group IX			3.1883 (0.6597–15.4090)	> 0.15
37.5 μg Hg within first 6 months	7 (77.78)	640 (52.33)		
0 μg Hg within first 6 months	2 (22.22)	583 (47.67)		

[a] These controls had to be continuously enrolled from birth for at least 7.13 years (mean age of initial diagnosis of emotional disturbances plus the standard deviation of mean age of initial diagnosis of emotional disturbances).
Hg, mercury; μg,= microgram.

Table 4. A summary of the logistic-regression results of the effect of Hg exposure from Thimerosal-containing hepatitis B vaccine administration within the first 6 months of life for the cases diagnosed with emotional disturbances in comparison to controls.[a]

Group examined (ICD-9 Code)	Reference dose 0 μg Hg (%)	12.5 μg Hg (%)	25 μg Hg (%)	37.5 μg Hg (%)	Odds ratio per μg Hg (95% CI) [*p*-value]
Emotional disturbances cases (313.xx)	15 (2.90)	7 (1.36)	456 (88.20)	39 (7.54)	1.025 477 (1.008 195–1.043 055) [< 0.005]
Controls	1143 (4.16)	542 (1.97)	24 554 (89.32)	1252 (4.55)	

[a] These controls had to be continuously enrolled from birth for at least 7.13 years (mean age of initial diagnosis of emotional disturbances plus the standard deviation of mean age of initial diagnosis of emotional disturbances).
Hg, mercury; μg, microgram.

Discussion

The present prospective, longitudinal case-control epidemiological study was undertaken to evaluate the potential relationship between increasing doses of Hg from Thimerosal-containing hepatitis B vaccines administered at specific times within the first 6 months of life and the long-term risk of an individual being diagnosed with an ED. It was observed that cases diagnosed with an ED were significantly more likely to receive increased Hg exposure from Thimerosal-containing hepatitis B vaccines administered within the first month of life, the first 2 months of life and within the first 6 months of life. The data were then separated by gender and the effects remained significant for males, but not for females. Finally, it was observed from logistic regression analysis that, among cases diagnosed with ED in comparison to controls, there was a significant increasing dose-dependent relationship between increasing Hg exposures from Thimerosal-containing hepatitis B vaccines administered within the first 6 months of life.

The results observed in the present study are consistent with a previous ecological epidemiological study of medical records within the VSD database [10]. It was observed in this previous study that there was a significant dose-dependent relationship between increasing Hg exposure from Thimerosal-containing vaccines administered within the first 7 months of life (rate ratio = 2.27 for a 100 μg Hg difference) and within the first 13 months of life (rate ratio = 2.91 for a 100 μg Hg difference) and EDs.

The results observed in the present study are also consistent with a number of previous clinical case-series studies revealing that occupational Hg exposure in adults was associated with significant emotional problems. For example, investigators reported on comprehensive emotional functioning of a group of 13 workers exposed to inorganic Hg vapour over a 2–4-week period compared to a normal control group [11]. The investigators observed among the Hg-exposed workers emotional problems, including: increased focus on physical functioning, depression, anxiety and social withdrawal. Similarly, other investigators described emotional problems in 15 workers poisoned by inorganic Hg [12]. Of the 15 subjects in the study, these investigators observed that nine were highly affected, five were moderately affected and one was mildly affected. The authors explained that the subjects reported impaired family relationships, irritability, impulsivity, social anxiety, angry outbursts and psychological pain. The extent of social anxiety was expressed by feelings such as wanting to 'run away from' and avoid others, including their families. They also reported sadness, difficulty sleeping and impaired concentration. These finding has been corroborated by other studies that find workers with chronic Hg exposure consistently show effects related to anxiety, depression and phobic avoidance [13].

Moreover, it was previously reported that the cumulative dose of Hg from Thimerosal-containing vaccines and environmental Hg exposure resulted in some infants receiving doses of Hg in excess of the US Environmental Protection Agency (EPA), the US Food and Drug Administration (FDA), the CDC and World Health Organization (WHO) Hg safety limits [6]. In addition, other investigators observed that administration of Thimerosal and other Hg compounds to various animal model systems resulted in symptoms consistent with those observed in an ED diagnosis, particularly among males [14–16].

The results of the present study stand in contrast to a single previous epidemiological cohort study of Thimerosal-containing vaccines in the VSD database [17]. In this previous study, the investigators described overall that they failed to find consistent significant relationships between increasing Hg exposure from

Thimerosal-containing vaccines administered at specific times within the first 7 months of life and the long-term risk of a child being diagnosed with neurodevelopmental disorders; and they also failed to find any significant relationships between increasing Hg exposure from Thimerosal-containing vaccines administered at specific times within the first 7 months of life and the long-term risk of a child being diagnosed with an ED. However, this study was marred by the lack of follow-up of the cohort of children examined. As a consequence, they potentially missed significant relationships between increasing Hg exposure from Thimerosal-containing vaccines and the long-term risk of children being diagnosed with neurodevelopmental disorders. An appropriate length of follow-up of individuals within the VSD database for neurodevelopmental disorder outcome is of paramount importance in order to find the true potential relationships between Hg exposure from Thimerosal-containing vaccines and neurodevelopmental disorder outcomes. For example, in a cohort study, inadequate follow-up means that many individuals will not be detected as having the outcome under study because they were not followed for a long enough period to receive a diagnosis. In the case of a case-control study, inadequate follow-up means that many individuals selected as controls will not be detected as having the outcome under study because they were not followed for a long enough period to receive a diagnosis.

To examine the potential impact of inadequate continuous follow-up time from birth in the present study, it was decided to reduce the length of continuous follow-up time from birth among the controls examined in the present study. As shown in Table 5, by reducing the length of continuous follow-up from birth among controls to 5.33 years (mean age of initial diagnosis of ED) from 7.13 years (mean age of initial diagnosis of ED plus the standard deviation of mean age of initial diagnosis of ED), the effect was no longer observed. The cases diagnosed with an ED in comparison to controls were at no increased ORs for exposure to Thimerosal-containing hepatitis B vaccines (administered within the first month of life, the first 2 months of life or the first 6 months of life). These observations in Table 5 are consistent with those described in the previously mentioned cohort study in the VSD database [17], but stand in stark contrast to those observed in Table 1 in the present study or those observed in the previous ecological epidemiological study of medical records within the VSD database where methods were utilized to ensure appropriate outcome status of the cohorts examined [10].

It is not surprising that the present study results indicate that there are gender differences in the effects of Thimerosal relative to the risk of an ED diagnosis, with males showing a greater effect. Many previous studies indicate that males are more significantly impacted by Thimerosal exposure than females [14,15,18–25].

For example, a mouse model system revealed that males were ~ 3-fold more susceptible to Thimerosal-induced neurotoxic effects and death than females [20]. As another example, the effects of neonatal treatment of rats with Thimerosal on behaviours, such as locomotor activity, anxiety, social interactions, spatial learning and on the brain's dopaminergic system were examined [14]. It was determined that, even though both genders manifested impairments, males were more sensitive than females to the neurodisruptive/neurotoxic actions of Thimerosal. In addition, epidemiological studies in humans also suggest that males are more vulnerable to Thimerosal toxicity [21,24,25]. For instance, two CDC-sponsored studies conducted by Thompson et al. [22] and Barile et al. [23] reported an association between Thimerosal exposure during the first 7 months of life and the presence of tics in boys.

Finally, in considering the apparent gender differences observed in this study regarding Thimerosal exposure and the risk of an ED diagnosis, it should also be considered that a relatively small sample of female cases diagnosed with an ED were examined. This occurred because there was a preponderance of male cases diagnosed with an ED relative to female cases diagnosed with an ED (male/female ratio = 3.4). As a result, this study may have been statistically underpowered to fully reveal the potential relationship between Thimerosal-containing vaccine exposures and the risk of an ED diagnosis. Future studies should examine larger populations of females diagnosed with an ED to determine if there is a significant relationship between Thimerosal-containing vaccine exposure and the risk of an ED diagnosis.

Study limitations

It is possible that the results observed may have occurred from unknown biases of confounders present in the datasets examined. However, several studies that have investigated the possible link between Thimerosal-containing hepatitis B vaccine exposure and neurodevelopmental disorder outcomes in the VSD database [24,25] found that outcomes not biologically plausibly linked to post-natal Hg exposure from

Table 5. A summary of exposure to Hg from Thimerosal-containing hepatitis B vaccine administration among cases diagnosed with emotional disturbances in comparison to controls[a] within the VSD database.

Group examined	Number of cases diagnosed with emotional disturbances (%)	Number of controls without an emotional disturbances diagnosis (%)	Odds ratio (95% CI)	*p*-value
Exposure Group X			1.0351 (0.8671–1.2357)	> 0.70
12.5 µg Hg within 1st month	204 (39.46)	22 897 (38.64)		
0 µg Hg within 1st month	313 (60.54)	36 365 (61.36)		
Exposure Group XI			1.0427 (0.8729–1.2456)	> 0.60
25 µg Hg within first 2 months	205 (40.20)	22 920 (39.19)		
0 µg Hg within first 2 months	305 (59.80)	35 558 (60.81)		
Exposure Group XII			1.1931 (0.6563–2.1692)	> 0.60
37.5 µg Hg within first 6 months	39 (72.22)	4 428 (68.54)		
0 µg Hg within first 6 months	15 (27.78)	2 032 (31.46)		

[a] These controls had to be continuously enrolled from birth for at least 5.33 years (mean age of initial diagnosis of emotional disturbances).
Hg, mercury; µg, microgram.

Thimerosal-containing hepatitis B vaccines (e.g., failure to thrive, cerebral degenerations, congenital anomalies, or febrile seizures) do not show significant positive associations between them and increasing Hg exposure from Thimerosal-containing hepatitis B vaccines (using the same methods of analyses employed to examine neurodevelopmental disorder outcomes).

In addition, when further considering the aforementioned potential limitation, the present study retrospectively examined automated medical records of patients HMO-enrolled from birth that were prospectively collected. The study design employed in the current study ensured that all individuals evaluated were followed on a prospective longitudinal basis from birth without knowledge as to their long-term diagnostic status. The exposures examined in the current study occurred early within the post-natal period and the ED outcome examined was not made for many months or many years after the exposures occurred. As a consequence, healthcare providers would not have associated exposure status with the outcomes examined. Further, it is of importance to consider that potential differences in healthcare availability among cases/controls were minimized, because all individuals examined were continuously HMO-enrolled from birth, so availability to healthcare was equal for everyone studied.

Another potential limitation of the present study was that there may be differences between the VSD population examined and the US population that could limit the generalizability of the VSD findings. However, investigators recently examined this potential limitation in the VSD database [26]. They determined that the VSD population is representative of the general US population on several key demographic and socioeconomic variables. These investigators also concluded that, despite a few specific groups being under-represented in the VSD compared to the US, the absolute number of VSD members is large enough to ensure significant representation of these groups in vaccine safety studies that use VSD data.

An additional potential limitation to consider is the reason for the differences in cumulative doses of Hg exposure that the children received at specific intervals within the first 6 months of life. The differences in exposure to Hg from Thimerosal-containing hepatitis B vaccines were not the result of a small group of children receiving anomalously high exposure, but instead may have resulted from the wide-ranging recommendations for routine hepatitis B vaccination during the 1990s. This is supported by the fact that the Advisory Committee on Immunization Practices (ACIP) in 1991 recommended that infants receive their first dose between birth and 2 months of age, their second dose between 1–4 months of age and their third dose between 6–18 months of age [27].

The present study also suffers from the potential limitation that additional analyses were not undertaken to evaluate Hg exposure from all Thimerosal-containing childhood vaccines. This was not possible because of the way in which the CDC assembled the VSD datasets. Specifically, the CDC assembled VSD datasets so as to provide information on only one type of vaccine per VSD dataset, and it was not possible to join separate VSD datasets. The ability to examine additional precise-timing and cumulative dosing phenomena would be worthwhile in future studies. It would also be worthwhile in future studies to examine the potential impact that covariates such as race, birth weight, parental mental illness, socioeconomic status, genetic susceptibility, etc. may have on the magnitude of the adverse effects observed. It would also be interesting to see what, if any, impact matching cases and controls on year of birth would have on the results observed.

Conclusion

The present study provides new epidemiological evidence significantly associating increasing Hg exposure from Thimerosal-containing hepatitis B vaccines administered at specific intervals within the first 6 months of life and the long-term risk of a child being diagnosed with an ED. In addition, it was observed that the effects observed were dose-dependent and, when separating the data by gender, the effects of increasing Hg exposure from Thimerosal-containing vaccines were significant among males, but not females. The results observed in the present study are supported by previous epidemiological/clinical studies showing a significant relationship between Hg exposure and ED diagnoses and animal model systems of Hg-induced ED symptoms. In considering the results of the present study, it is important to realize that routine childhood vaccination is a significant public health tool to reduce the morbidity and mortality of infectious diseases [28], but there is also an urgent public health need to remove the unnecessary use of Thimerosal in vaccines as soon as possible.

Declaration of interest

Three of the four authors have been involved in vaccine/biologic litigation. This study was supported by the non-profit Institute of Chronic Illnesses, Inc. and the non-profit CoMeD, Inc.

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