1st Scientific Session of the International Clearinghouse for Birth Defects Surveillance and Research (ICBDSR)

Epidemiology and Prevention of Birth Defects

Evaluation of Medications as Teratogens

Abstracts

September 18-19th 2005
MALTA
ICBDSR SCIENTIFIC SESSION – MALTA 18-19TH SEPTEMBER, 2005

18th September, 2005, 9:00 a.m. – 4:30 p.m.

Opening Address: Dr. Ray Busuttil, Director General Health, Malta

Welcome: John Harris, California Birth Defects Monitoring Program, Berkeley, US.

Morning Session: Evaluating Medication Exposures and Birth Defects (Chair: J. Harris)

1. “Maternal Progestin Risk and Hypospadias” Suzan Carmichael, California Birth Defects Monitoring Program, Berkeley, US.

2. “Components of Pharmacovigilance in Relation to Birth Defects” Helen Dolk, University of Ulster, Newtonabbey, Northern Ireland.

3. “Patterns of Prescription Drug Use Before, During, and After Pregnancy in Relation to Type of Drug and Fetal Risk” Lolkje T. W. de Jong – van den Berg, Groningen University Institute for Drug Exploration (GUIDE), Groningen, Netherlands.

- BREAK - 10.30 – 11.00 a.m

4. “Genetic Variation in Detoxification, Exogeneous Exposures and Risks of Selected Birth Defects” Gary Shaw, California Birth Defects Monitoring Program, Berkeley, US.


- LUNCH - 12:30 – 2:00 p.m

Afternoon Session: Update on Anticonvulsants Teratogenicity (Chair: E. Robert-Gnansia)


2. “Long Term Outcome of Children Born to Mothers with Epilepsy” Naghme Adab, The Walton Center for Neurology and Neurosurgery, Liverpool, UK.

- BREAK - 3.00-3.30 p.m

3. “Valproic Acid Teratogenic Effects: Old Problem, New Approach” Valentina Massa, Department of Biology, University of Milan, Milan, Italy.

Morning Session: **Evaluating Drugs and Birth Defects (Chair: P. Mastroiacovo)**

**Keynote:** “The ICBDSR MADRE Project for Evaluating Drugs in Pregnancy in Birth Defects Registries: Past, Present and Future” Pierpaolo Mastroiacovo, ICBDSR Centre Director, Rome, Italy.

**Other Presentations:**


3. “Birth Defects’ Occurrence in Offspring of Mothers Taking 1st Trimester Medication in the Czech Republic” Jiri Horacek et al. Chair of Medical Genetics, Postgraduate Medical Institute, Prague, Czech Republic.


- **BREAK – 10.30 – 11.00 a.m**

5. “Birth Defects and Drug Exposure Surveillance in the Northern Netherlands” Marian Bakker et al. EUROCAT Northern Netherlands, Department of Medical Genetics, University Medical Centre, Groningen, Netherlands.

6. “The Frequency and Severity of Neonatal Abstinence Syndrome in Infants Born to Selective Serotonin Reuptake Inhibitor Treated Mothers” Gil Klinger et al. Department of Neonatology, Rabin Medical Center, Petah Tiqva, Israel.


**REVIEW OF POSTERS AND CLOSING ADDRESS**
ORAL PRESENTATIONS:

EVALUATING MEDICATION EXPOSURES AND BIRTH DEFECTS (CHAIR: J. HARRIS)

Maternal progestin intake and risk of hypospadias.


Introduction
Previous studies have suggested that maternal intake of progestins during early pregnancy may be associated with an increased risk of hypospadias. Progesterone and its derivatives are commonly prescribed during early pregnancy, for example in cases of luteal phase dysfunction and in conjunction with ovulation stimulation drugs. This study examined whether risk of hypospadias was associated with periconceptional progestin intake.

Methods
This study includes data on deliveries with estimated due dates from October, 1997 to December, 2000 that were part of the National Birth Defects Prevention Study, a population-based, multi-state case-control study. The analysis included 502 cases diagnosed with second or third degree hypospadias (i.e., the urethra opened at the penile shaft, scrotum or perineum) and 1286 male, liveborn, non-malformed controls.

Results
Forty-two case mothers (8.4%) and 31 control mothers (2.4%) reported any pregnancy-related progestin intake from four weeks before through fourteen weeks after conception, resulting in an odds ratio (OR) of 3.7 (95 percent confidence interval (CI) 2.3-6.0). Analyses stratified by several potential covariates also suggested elevated risks. For example, among the 10 cases and 13 controls who did not report any fertility-related procedures or treatments other than progestins, the odds ratio was 2.2 (95 percent CI 1.0-5.0). Progestin intake for the purpose of contraception was not associated with increased risk.

Conclusions
This study found that pregnancy-related intake of progestins was associated with increased hypospadias risk.
Using registries of congenital anomaly for postmarketing drug surveillance

Helen Dolk, EUROCAT Project Leader, University of Ulster
Lolkje de Jong van den Berg, Willemijn Meijer, University of Groningen.

The questions that surveillance addresses in relation to teratogenic effects of drugs are broadly the following: 1) Does a new drug on the market carry a previously unknown risk of malformation if used in pregnancy? 2) Does an old drug on the market carry a previously unknown risk of malformation if used in pregnancy? 3) Are known teratogenic drugs or possibly teratogenic drugs being used despite warnings, particularly before pregnancy is recognized? 4) Are the least teratogenic drug options being used for chronic conditions (e.g. epilepsy) during pregnancy? 5) Are warnings to avoid pregnancy in case of necessary use of known teratogenic drugs successful?

Congenital anomaly registries are essential components of the surveillance strategy to address these questions, alongside prospective pregnancy studies and follow-ups of exposed cohorts. Registries need to make some key initial decisions on whether to collect data on drugs routinely, and if so whether to collect data on non-malformed controls, or to use the registry as a basis for ad-hoc case and case-control studies within a surveillance framework, particularly to test emerging hypotheses.

EUROCAT covers 30% of the birth population of the European Union, 1.2 million births per year. In 2004, EUROCAT undertook a survey of registry practice in relation to recording of drug exposure. 31 out of 40 registries collect drug data routinely but only 4 collect information for non-malformed controls. The main sources of information were obstetric data and maternal interview data, and only one had linkage to a population-based pharmacy database.

The central database 1996-2000 was analysed relating to 19,537 cases from 16 registries. The proportion of cases with recorded drug exposure varied from 5 to 26%, and when drugs were classified to 26 categories, it was clear that different registries “specialised” in different drug categories, either because of their data sources or because of differences in drug prescribing or taking behaviour.

An investigation of the hypothesis that loratidine exposure may be associated with hypospadias was undertaken. The results were inconclusive, mainly because underascertainment of exposure greatly reduced study power for this uncommon exposure. The study highlighted the benefit of linkage to a pharmacy database.

Networking of registries is essential to achieve sufficient population size in postmarketing drug surveillance. Further linkage of registries to pharmacy databases and to clinical cohorts would greatly enhance their potential for surveillance. A clearer surveillance structure at European level in terms of funding and the systematic generation and testing of hypotheses would make optimal use of the potential of congenital anomaly registries.
Patterns of Prescription Drug Use Before, During and After Pregnancy in Relation to Type of Drug and Fetal Risk

Lolkje T. W. de Jong-van den Berg,
Groningen University Institute for Drug Exploration (GUIDE), Groningen, Netherlands.

The thalidomide disaster in the early 1960s prompted major changes in the scope and authority of drug regulatory agencies around the world. Despite these changes, there remains no systematic approach to the identification of new teratogens. Current controversies over the SSRIs in children have led to calls for additional changes in postmarketing approaches, but little attention is focused on improving postmarketing surveillance for new teratogens, a problem identified over four decades ago.

Every year new drugs are approved and marketed. However, women who might become pregnant are excluded, particularly if there is any suspicion generated from animal studies that a drug might be teratogenic. So knowledge about the safety of drug use during pregnancy comes available only in the post-marketing setting, and of course, only after the drug has been used by pregnant women. In most countries their effects on reproductive outcomes are not reported or utilised in any systematic way.

A comprehensive surveillance system to identify teratogens is critically needed. Two main study approaches have been developed for the purpose of identifying teratogens in the post-marketing setting: follow-up and case-control studies. Follow-up studies identify women exposed to specific drugs and followed to identify the outcomes of their pregnancy. These pregnancy registries are efficient to identify high-risk teratogens (an example is isotretinoin) but the relatively small samples recruited in these registries provide insufficient power to identify moderate-risk teratogens.

In case-control studies subjects are selected on basis of whether they do (cases) or do not (controls) have a particularly birth defect under study. The groups are then compared with respect to the proportion having or not having be exposed to the drug of interest. A major strength of case-control studies is their substantial power (example DES in utero and vaginal cancer). The birth defect registries (as ICBDSR and EUROCAT) have the opportunity to examine specific defects in relation to the wide range of medications taken by pregnant women. This case-control design requires, however, complete and valid information with respect to antenatal drug exposure (prescription drugs and over-the-counter drugs). Different sources of drug information can be used: obstetric records, interviews with the mother or pharmacy data.

Misclassification of exposure can lead to substantial bias, and therefore drug utilisation studies in pregnant women can be very informative. In North Netherlands we have a population based pregnancy database with detailed information about prescription drugs originated from pharmacies. From this database we know that 79% of pregnant women received at least one prescription drug during their pregnancy. The prescription rate increased from 44 per 100 women in the first trimester to 49 and 61 per 100 women in the 2nd and 3rd trimester of pregnancy (p=0.000) of all pregnant women, and if vitamins and dietary supplements are included, almost all pregnant women use any drug during pregnancy. Information about the exposure to specific drug groups in the general population of pregnant women is essential to know whether the exposure in case-control settings is reasonable. Pharmacy data are prospectively recorded and therefore an unbiased source of drug exposure information in case-control settings. During the presentation figures of the use of specific drug groups before, during and after pregnancy in relation to type of drug and the foetal risk will be presented.

Prof. dr. Lolkje TW de Jong-van den Berg
Department of Social Pharmacy, Pharmacoepidemiology and Pharmacotherapy.
Groningen University Institute for Drug Exploration (GUIDE), University of Groningen, The Netherlands
Genetic variation in detoxification or homocysteine metabolism, exogenous exposures, and risks of selected birth defects.

Shaw GM, Jovannisci DM, Carmichael SL, Yang W, Finnell RH, Lammer EJ.

The combination of genetic susceptibility and environmental exposure likely contributes substantially to the occurrence of birth defects in humans. Until recently, however, the genetic methods that would enable the exploration of genetic susceptibility to exposures among large human populations were unavailable. We have been exploring whether genetic variation of infant genes involved in various biochemical pathways, including biotransformation of endogenous nonpeptide compounds, xenobiotic detoxification, or homocysteine metabolism, modify risks of malformations in the presence or absence of selected maternal exposures to xenobiotics. Detoxification of drugs and environmental compounds is carried out by a group of drug metabolizing enzymes. Although mainly known as “drug metabolizing enzymes,” these enzymes are involved in metabolizing both endogenous compounds and myriad xenobiotic chemicals. For endogenous compounds, these enzymes regulate the synthesis and degradation of nonpeptides such as ligand-modulated transcriptional factors, including second messenger pathways, that influence the basic embryologic processes of growth, differentiation, apoptosis, cell migration and neuroendocrine functions. For xenobiotics, these enzymes are important for detoxifying both parent compounds and reactive intermediate chemicals that may be teratogenic. These drug metabolizing enzymes are divided into Phase I and II enzymes, based upon the types of biochemical reactions that they catalyze. We have genotyped hundreds of case and control infants for the following detoxification genes: NAT1 1088, NAT1 1095, NAT2, GSTT1, GSTM1, CYP2C19, CYP2E1, CYP3A4; EPHX1, GSTP1, and NQO1. We have investigated gene-only effects for NTDs and orofacial clefts. We have also investigated gene-exposure interaction effects where the exposures have included maternal cigarette smoking, occupational chemicals, and maternal use of multivitamins. With respect to homocysteine, we have genotyped hundreds of cases and controls for the following genes involved in the homocysteine/folate biochemical pathway: NOS3, MTHFR, and RFC1. For these genes, we have investigated gene-only effects for orofacial clefts and conotruncal heart defects. In addition, for these genes we have investigated gene-exposure interaction effects where the exposures have included maternal cigarette smoking and maternal use of multivitamins. Our presentation will describe many of these results and will highlight some of the important methodologic considerations associated with these types of “molecular” epidemiology investigations.
The California BioBank – a Resource to Evaluate Medication and Other Exposures and Birth Defects

John Harris,
Chief, California Birth Defects Monitoring Program, Berkeley, CA.

The California Birth Defects Monitoring Program is now banking mid-pregnancy blood samples on 110,000 women per year, the largest resource of its kind in the United States.

The California Birth Defects Monitoring Program (CBDMP) was founded in 1982 by Dr. John A. Harris with a mission to find causes of birth defects. The CBDMP collects different kinds of data to test hypotheses about causes. Over the past twenty years, CBDMP has developed the capability to design and conduct large population-based studies by abstracting medical charts from mothers and babies, interviewing cases and controls and by running genetic tests on newborn blood spots.

In February 2003, the California Birth Defects Monitoring Program began to bank pregnancy blood samples and to link these biologic samples with birth outcomes. As of August 2005, CBDMP has banked over 207,000 pregnant women’s blood samples. This biobank is growing at the rate of 110,000 pregnant women per year.

Among a large, racially diverse, near population-based sample, the pregnancy blood sample bank allows for direct measurement of infectious antibodies, medications, nutritional, chemical, lifestyle, metabolic and genetic exposures. These exposures can be linked to birth outcomes to study causes of birth defects, prematurity, mental retardation and other pediatric diseases.

We invite scientists of many disciplines to use our biobank to directly measure many types of exposures. For further information, please contact Dr. John A. Harris: jha@cbdmp.org; 1917 5th Street, Berkeley, CA 94710, phone: 510.849.5841.
UPDATE ON ANTICONVULSANTS’ TERATOGENICITY (CHAIR: ELISABETH ROBERT-GNANSIA)

Introduction

Elisabeth Robert-Gnansia, Lyon, France

Since the first recognition of facial dysmorphia in children born to mothers treated with phenobarbital or phenytoin, a huge amount of literature has been published on the effects of antiepileptic drugs on pregnancy outcome. There has long been a consensus among experimental and clinical teratologists that anticonvulsants are teratogenic. Now a number of questions remain to be solved. The most crucial one is: how to treat epileptic women in childbearing age in order to avoid seizures without harming their children? Other important questions are: why are anticonvulsants teratogenic, what is their mechanism of action? are all women equally sensitive to the teratogenic effect of anticonvulsants? are newly marketed anticonvulsants safer than the classical ones? Are structural and visible malformations the only unwanted effects of anticonvulsants on in utero exposed children?

This workshop has been set up in an attempt to clarify these points and speakers have been selected among the most outstanding scientists in the world with the hope to help researchers, advisers, and clinicians better managing epilepsy during pregnancy.
Gene-environment interactions in the etiology of anticonvulsant drug-induced birth defects.

Richard H. Finnell, Bogdan Wlodarczyk, Leeyean Wong, Huiping Zhu and Robert Cabrera,

Center for Environmental and Genetic Medicine, Institute of Biosciences and Technology, Texas A&M University Health Science Center, Houston, Texas  77030, USA.

It is widely accepted that most all of the front-line anti-epileptic medications (AEDs) have a well documented teratogenic potential, resulting in a small percentage of the exposed offspring to present with a recognized pattern of both major and minor malformations. The affected infants may have craniofacial dysmorphia, cardiac anomalies, limb and neural tube defects. Like most human teratogens, the mechanism by which these compounds exert their adverse effects on developing systems is not well understood. Not all infants exposed to environmental toxicants are equally susceptible to the induction of birth defects. Understanding the genetic regulation of susceptibility to teratogenesis at the molecular level is an essential first step in preventing the birth of infants with AED-induced birth defects.

Our laboratory has developed mouse models using valproic acid (VPA) and phenytoin (PHT) that illustrate the inherent complexity involved in trying to unravel gene-environment interactions in order to better understand the genetic basis of susceptibility to these AEDs-induced congenital anomalies. The malformations that will be considered include neural tube defects (NTDs), conotruncal heart defects and craniofacial anomalies. Using mouse models that rely upon genetic variation among different inbred mouse strains, we have demonstrated differential sensitivity to AED-induced congenital malformations. Specifically, SWV is highly sensitive to VPA-induced NTDs, while C57 mice are resistant. On the other hand, C57 mice are relatively sensitive to ocular defects and clefting disorders induced by PHT, while the sensitivity to these disorders differs remarkably among C57, LM/Bc and SWV mice.

Several different hypotheses have been proposed explain the teratogenicity associated with AEDs. These include the generation of reactive oxidative species and drug-induced embryonic arrhythmia. Other possibilities include differences in the rate at which intermediary metabolites are eliminated, inhibition of folate metabolism, and interactions with glucocorticoid receptors. At the core of these mechanisms are genetic regulatory elements such that in utero exposure to AEDs alters the expression of essential genes that compromises normal embryonic development. Our laboratory has been actively seeking the gene or genes involved in regulating susceptibility to AEDs, using Depakene (valproic acid, VPA, Abbott Laboratories) and related molecules as a model compound. We have used whole genome scans, cDNA microarrays and other approaches to identify critical genetic elements regulating susceptibility to AED-induced congenital abnormalities in the mouse. The data obtained suggest both a fetal genetic contribution and a maternal effect, while the former being consistent with a phenotype that is inherited in an autosomal recessive pattern with incomplete penetrance. Haplotype analysis showed that a primary gene of interest has been mapped to between D7Mit285-D7Mit101. These results suggest a single major gene as the primary factor in determining sensitivity to VPA-induced NTDs in the mouse.

It is of interest that most of the clinically effective AEDs are also folic acid antagonists. Based upon recently obtained gene microarray data, it is apparent that the folate binding protein is involved in the tetrahydrobiopterin salvage pathway, functioning in the metabolic recycling of pterins to provide adequate amounts of co-factor for proper NOS activity. The cellular consequences of abnormal NOS functions are believed to contribute to the observed congenital malformations in the Folbp1 deficient knockout embryos. As a result of exposure to reactive oxidative species, embryos are subjected to increased rates of apoptosis and decreased cellular proliferation. Such abnormalities in cellular kinetics can potentially explain the pattern of defects observed in the affected neonates and perhaps, in human infants as well.

Supported in part by NIH grants ES ES09417, ES09106
The Longer Term Outcome of Children born to Mothers with Epilepsy.

Naghme Adab,
Walton Centre for Neurology and Neurosurgery. Liverpool, UK

Background
The risk of major malformations following antiepileptic drug exposure in pregnancy has been well-established for children of mothers with epilepsy. However the longer term outcomes of children exposed to antiepileptic drugs remain controversial.

There are several key questions:
1. How common is developmental delay or cognitive impairment?
2. Are dysmorphic features predictive of developmental delay?
3. Are there drug specific effects?
4. To what extent do genetic factors associated with epilepsy or seizures during pregnancy contribute to the outcomes observed?

Objectives
The evidence for the developmental outcomes of children exposed to antiepileptic drugs in utero will be discussed, with particular emphasis on the findings of a large retrospective study of children of mothers with epilepsy, who had detailed neuropsychological assessments. This study will be used to illustrate the complexity of this area of study.

Evidence from the current literature for drug specific effects and confounding factors will be discussed and the implications for practice will be explored.
Valproic acid teratogenic effects: old problem, new approach

Valentina Massa,
Department of Biology, University of Milan, Milan, Italy

Therapeutic management of women with epilepsy during the reproduction years requires maintaining a precarious balance between controlling seizures and minimizing potential fetal exposure to the harmful effects of medications.

Valproic acid (VPA) is an anticonvulsant drug widely used therapeutically for a variety of neurological conditions, including epilepsy. VPA is also well known for its teratogenic potential in both humans and experimental animal models. The typical malformations observed following VPA exposure include neural tube defects (NTDs), craniofacial and skeletal malformations. Nevertheless, the mechanisms underlying VPA’s anticonvulsant efficacy or its teratogenicity remain to be elucidated. In order to study valproic acid-induced teratogenic effects, two approaches were utilized. In the first project, gene-expression profiles were analyzed, whereas the second study was focused on histone acetylation status. Using anti-sense RNA amplification and cDNA microarrays, we examined the expression of approximately 5700 genes in the first six postotic somites of control and treated embryos at 6, 12, 18 and 24 hours after the 8.5 days post coitum (d.p.c.) VPA treatment (1.36 and 2.72 mmol/Kg). Analysis indicated that several ontological groups (histone deacetylase complex, GTPases, cell proliferation, apoptosis and cytoskeletal) have significantly enriched gene expression changes in response to the teratogenic insult.

The histone deacetylase (HDAC) enzymes participate in the nucleosome structure control. Several studies showed that VPA is a strong inhibitor of HDAC activity in cell and animal models, producing histone hyperacetylation. In order to better define the correlation between HDAC inhibition and teratogenicity, pregnant mice were treated i.p. on 8.5 d.p.c. with 400 mg/Kg VPA. One hour after treatment, embryos were processed for western blotting and immunohistochemical analysis, using antibodies anti hyperacetilated histone H4. VPA exposure in utero induced hyperacetylation of embryonic proteins, specifically those localized in the caudal neural tube and in the somites, the main target organs of VPA teratogenic effects.

The results of these works suggest that valproic acid induces congenital malformations through gene-expression alterations by chromatin structure misregulation.
Experience with new antiepileptic drugs in pregnancy.

Schaefer C.
Beratungs- und Pharmakovigilanzzentrum für Embryonaltoxikologie Berlin, Germany

New antiepileptic drugs (New AEDs) are felbamate (FBM), gabapentin (GPT), lamotrigine (LTG), levetiracetam (LEV), oxcarbazepine (OXC), pregabalin (PGB), tiagabine (TGB), topiramate (TPM), vigabatrin (VGB), and zonisamide (ZNS). In particular LTG, GPT, and TPM are frequently prescribed for partial and generalized seizures nowadays, also to women of reproductive age. General advantages of new AEDs are a) better tolerance and similar efficacy for partial epilepsy in monotherapy (LTG, TPM, GPT, OXC, VGB), b) less (TPM, OXC) or no cytochrome P450 enzyme induction with the risk of failure of oral contraceptives, c) no substantial anti-folate properties, d) less adverse effects on hormones and metabolism (adipositas, insuline resistance, polycystic ovary syndrome - PCOS etc), e) low protein binding, simple pharmacokinetics.

Apart from published data we present the outcome of exposed pregnancies ascertained by the Teratology Information Service (TIS) in Berlin. Up to now, we recorded 289 pregnancies exposed to new AEDs (GPT 40, LEV 14, LTG 183, OXC 21, PGB 4, TGB 1, TPM 19, VGB 7).

For evaluating specific teratogenicity prospectively ascertained cases with monotherapy allow a preliminary assessment of the relative risk for (major) birth defects (BD) whereas retrospective case reports may contribute to the search for specific pattern of anomalies. Apart from databases of TIS, most information on new AEDs is available through sponsor’s registries, and epilepsy & pregnancy projects.

With approximately 1,000 prospectively documented pregnancies under monotherapy, only the published and available unpublished data for lamotrigine (followed by gabapentin and oxcarbazepin) allow preliminary risk estimations. The risk of 2-3% found for major BD under lamotrigine monotherapy is not significantly increased compared to the known prevalence in the general population. No major or minor anomaly pattern has been detected so far.

Detailed dysmorphological exam or long term follow up for mental effects in infants prenatally exposed to newer AEDs has not yet been evaluated with the exception of very few observations.

Although data are insufficient to proof safety of one of the new AEDs, experience with monotherapy in pregnancy is promising. As there is growing concern about long term sequelae of valproic acid (VPA), one of the still frequently used classical AED, clinical teratologists should consider recommending those new AEDs, which seem to be less dangerous than VPA. This also implies reconsidering the broadly accepted recommendation not to change an effective AED regimen during (early) pregnancy or when planning a pregnancy.
EVALUATING DRUGS AND BIRTH DEFECTS (CHAIR: P. MASTROIACOVO)

Teratogenic drugs in pregnancy – an evaluation of routine data collection

Morgan Margery,
Congenital Anomaly Register & Information Service for Wales

Congenital anomaly data for Wales is recorded with details from maternal obstetric notes. In early pregnancy (booking) details are recorded about usage of medication. This is then reported to the congenital anomaly register once a case is notified.

In seven years of data collection, 1770 cases of maternal usage of medication were recorded from approximately 10,000 cases of congenital anomaly.

Of the medications recorded several drugs or families of drugs were classified as being a risk in the first trimester. The most common of these were anti-depressants of the selective serotonin re-uptake inhibitor group (SSRI). The associated anomalies were reviewed.
Use of Phenytoin, Phenobarbital, or Diazepam during Pregnancy and Risk of Congenital Abnormalities: a Case-Time-Control Study

Erzsébet H. Puhó, Dorte Kjær, Jakob Christensen, Mogens Vestergaard, Andrew Czeizel, Henrik Toft Sørensen, Jørn Olsen, Melinda Szunyogh, Júlia Ménteki, Csaba Siffel, János Sándor

Epilepsy affects 3-6 per 1,000 pregnant women, most of whom need medical treatment. Previous studies show that children exposed to antiepileptic drugs in fetal life have a 2.2 to 4.0-fold increased prevalence of congenital abnormalities, but teratogenic effects of individual drugs are not well known. Case-control studies are often used to examine teratogenic drug effects during early pregnancy (organogenesis) but these studies are subject to recall and participation bias. The case-time-control approach is less susceptible to these sources of bias. We illustrate this design in a study of congenital abnormalities following exposure to phenytoin, phenobarbital, or diazepam in pregnancy, using data from the Hungarian Case-Control Surveillance of Congenital Abnormalities (1980-1996). Among 22,843 cases and 38,151 controls, odds ratio (OR) for all congenital abnormalities was 1.2 (95 per cent confidence interval (CI): 1.0, 1.4) in children exposed to one of the three drugs. Among children exposed to diazepam, OR for all congenital abnormalities was 1.2 (95 per cent CI: 1.0, 1.4), OR for neural tube defects, 2.4 (95 per cent CI: 1.5, 3.7), OR for cleft lip with or without cleft palate, 1.8 (95 per cent CI: 1.1, 2.8), and OR for limb deficiencies, 2.6 (95 per cent CI: 1.3, 4.9). Our findings support a teratogenic effect of some of the drugs under study. The case-time-control design helps avoid some of the most serious sources of bias in case-control studies and should play an important role in future studies of rare side effects of drugs taken during pregnancy.
Birth Defects’ Occurrence in Offspring of Mothers Taking 1st Trimester Medication in the Czech Republic

Horacek, J.1,2, Sipek, A.3,4, Gregor, V.1,2, Masatova, D.5

1Postgraduate Medical Institute, Chair of Medical Genetics, Prague
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3Institute for Care of Mother and Child, Prague
4Postgraduate Medical Institute, Chair of Gynaecology and Obstetrics, Prague
5Institute of Health Information and Statistics, Prague

Objective
To study 1st trimester medication as a potential risk factor for birth defects.

Methodology

Results
There were 25,580 children born with a birth defects registered in the period under the study. Out of them, 1,004 were born to mothers taking 1st trimester medication making a total of 1,288 particular birth defects. A control group covered 1,321 exposed women giving birth to a child without any birth defect. A significantly higher risk was found for following defects: anencephaly, spina bifida, congenital hydrocephalus, anophthalmia/microphthalmia, some types of congenital hearth defects, cleft lip with cleft palate and limb reduction defects. A significantly higher risk was found also in following 5 types of drugs: anticoagulants, antihypertensivs, peripheral vasodilatants, urologics and antiepileptics.

Conclusions
Although the results are not always unambiguous and are probably influenced by both information and recall bias, they complement data on adverse effects of drugs in pregnancy in the Czech Republic. They also stress the need for a high preliminary caution in drug prescription and for a complex risk assessment.

This study was supported by an IGA MZ CR grant # 7516-3
Medication use during pregnancy in Hungary in recent years

Melinda Szunyogh, Erzsebet Puho, Csaba Siffel, Julia Metneki, Janos Sandor

Objectives
The purpose of our analysis was to evaluate the medication use during pregnancy among mothers of cases with birth defects and healthy controls in recent time period.

Methods
We used the population-based dataset of the Hungarian Case-Control Surveillance of Congenital Abnormalities to calculate the frequencies of drug intake by mothers during their pregnancy from 1997 through 2002. Cases with birth defects (n = 7,559) were identified from the Hungarian Congenital Abnormality Registry and standardized questionnaires were mailed to public health nurses who identified and matched two healthy control babies (n = 14,448) by gender, birth month, and maternal residential district, and interviewed mothers. We coded and grouped drugs according to the Anatomical Therapeutic Chemical (ATC) classification system. For statistical analysis, we applied logistic regression.

Results
86% of pregnant women (mean maternal age = 26.7 years) used some kind of medications. Medication use among younger pregnant women (<25 years) was higher compared to older maternal age group (≥25 years) (OR: 1.21, 95%CI (1.12-1.32)). Vitamins, mineral supplements, and antianemic preparations, which were used by the majority of mothers among both cases and controls (~76% any time during pregnancy; ~38% during the first trimester), drug use during pregnancy was higher (OR = 1.56; 95%CI = 1.47-1.65) among case mothers (49%) compared to control mothers (38%). Medication use was lower among both case (~19%) and control (~13%) mothers during the first trimester in comparison with intake during the second and third trimester (28%; 23%, respectively). The most frequently used therapeutic drugs were the following: antibacterials for systemic use, gynaecological anti-infectives and antiseptics, sex hormones and modulators of the genital system, drugs for obstructive airway diseases, and antihistamines for systemic use.

Conclusions
Our analysis showed that large proportion of pregnant women use medications in Hungary in recent years, which is higher among mothers of cases with birth defects compared to other European published figures, and also higher compared to the general pregnant population in Hungary represented by mothers of healthy control babies in the dataset. However, this proportion is lower than the previously reported figures for earlier time periods in Hungary.
Birth defects and drug exposure surveillance in the Northern Netherlands

M.K. Bakker¹, W.M. Meijer², H.E.K. de Walle¹

¹EUROCAT Northern Netherlands, Department of Medical Genetics, University Medical Centre Groningen.
²Department of Social Pharmacy, Pharmacoepidemiology and Pharmacotherapy, University Institute for Drug Exploration (GUIDE), Groningen, The Netherlands

Aim
To conduct a survey on maternal drug use in the 1st trimester and the occurrence of birth defects in a population based registry of congenital malformations in order to detect possible new teratogens.

Methods
We selected 3286 cases born between 1981 and 2003. Birth defects were coded according to ICD9 and ICD10. Drugs were coded according to the ATC-codes. We investigated combinations of 51 categories of malformations, not part of a chromosomal or monogenic disorder, with >= 10 subjects present and 92 drugs and groups of drugs with >= 10 exposed subjects present. As controls we used 669 subjects with a recognised chromosomal or monogenic disorder. For malformation-drug combinations with >= 2 exposed cases we measured the possible disproportionality by calculating the Chi² and the Reporting Odds Ratio (ROR) with a 95% Confidence Interval (CI).

Results
In total 656 malformation-drug combinations had >=2 exposed cases. For 24 combinations an increased occurrence was found with a p-value<0.01. Of these combinations 9 had a lower CI of the ROR >=3: VATER/VACTERL x paracetamol combinations (N02BE51); malrotation of intestins x benzodiazepine derivates (N05BA); VATER/VACTERL x benzodiazepine derivates (N05BA); omphalocele x ibuprofen (M01AE01); reduction defects of the upper limb x beta lactamase sensitive penicillins (J01CE); microcephaly x trimethoprim (J01EA01); Hirschsprung x hydrocortisone (D07AA02). For another 2 combinations the ROR could not be calculated because there were no exposed controls: ASD x diazepam (N05BA01); coarctation of the aorta x carbamazepine (N03AF01).

Discussion
The combinations of drugs and malformations that are disproportionate present in our database may reveal signals of potential teratogenic drugs. However, the most strong signals are not described in literature before. Therefore these results have to be interpreted carefully and critically. They have to be further evaluated, for example in an other database or by using analytic study designs.

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The frequency and severity of neonatal abstinence syndrome in infants born to Selective Serotonin Re-uptake Inhibitor treated mothers

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Context
Because selective serotonin reuptake inhibitors (SSRIs) are considered relatively safe, their use for treatment of depression during pregnancy has gained popularity. However, a number of reports have linked in utero exposure to SSRIs to a neonatal abstinence syndrome (NAS).

Objective
To examine the incidence and clinical characteristics of NAS in infants exposed in utero to SSRIs compared to non-exposed infants.

Design
Cohort study.

Setting
Tertiary-care university-affiliated center

Patients
One-hundred twenty term infants, of whom sixty had been exclusively exposed in utero for a prolonged period to SSRIs, including paroxetine, fluoxetine, citalopram, sertraline, and venlafaxine.

Outcome Measures
NAS was assessed by the Finnegan score, as follows: score of 8 or above – withdrawal syndrome; score of 4-7 -- mild ; and score of 0-3 -- normal. All infants were followed by a standardized protocol that included repeated assessments of the Finnegan score and cardiorespiratory monitoring until normalization of the Finnegan score.

Results
Of the 60 infants exposed in utero to SSRIs, 8 showed signs of a withdrawal syndrome and 10 exhibited mild symptoms. All control infants had a normal Finnegan score. In infants who developed withdrawal symptoms, the maximum mean (daily) Finnegan score was recorded within the first 48 hours of life, although in some infants maximal individual scores were recorded up to 4 days after birth. Three infants exposed to SSRIs for the complete pregnancy duration had major congenital anomalies (ventricular septal defect and cleft palate, ventricular septal defect, and hydronephrosis with ureterocele) vs, one infant in the control group (hydronephrosis).

Conclusions
NAS occurs in 30% of infants exposed in utero to SSRIs. These infants should be closely monitored for at least 48 hours after birth. The long-term effects of prolonged in utero exposure to SSRIs, particularly in infants who develop severe symptoms, have yet to be determined.
Is the Prevalence of Malformed Infants Related to Maternal Drug Exposure High in Japan?

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Objective
To investigate the prevalence of malformed infants related to maternal drug exposure is high or not in Japan.

Designs
A retrospective survey about medication among pregnant women who delivered infants with congenital malformations in Japan from 1997 to 2003, using the data from JAOG (Japan Association of Obstetrician and Gynecologists) Birth Defects Registry and the research database of our birth defects monitoring center. JAOG Birth Defects Registry is a nationwide hospital-based, covering about 10\% of whole births in Japan.

Main outcome measures & Results
During this period, 651,722 infants were monitored and 10,145 cases of congenital malformed infants were identified among them. Six hundred and forty six women had medication during their pregnant periods, and more than 150 types of drugs were prescribed. Most frequent drugs were tocolytic agents, anti-epileptic agents, and anti-thyroid agents. There were several malformed cases which were assumed to be related to the drugs taken in pregnant period.

Conclusion
The prevalence of malformed infants related to maternal drug exposure is not high ratio, but still we need to consider effective system which would prevent malformations related to maternal drug exposure.
POSTER PRESENTATIONS -

The true prevalence of congenital malformations in the Swedish population.

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The aim of the present study was to find out the quality of the Swedish Registry of Congenital Malformations (MBR). By studying the rate of some congenital malformations in terminated pregnancies (ToP) as well as in new-born children in six different registries and by combining those data it was possible to come very close to the true prevalence. The following congenital malformations were studied: Neural tube defects [spina bifida (MMC), anencephaly (AE) and encephalocele (EE)]; Trisomy 21, Down syndrome (DS); Cleft lip (CL) and cleft lip and palate (CLP).

The reporting rate of congenital malformations in new-born children to the MBR was high, about 80% for MMC, AE, CL and CLP and 75% for DS. The reporting rate in terminated pregnancies was, however, less good, 66% for MMC and 50% for DS.

The prevalence of MMC in Swedish new-born children reported to MBR is low 2.5/10 000 births. However, when ToP are included the prevalence is 5.5-6.0/10 000 births. When also missed cases from the other registries are calculated the true prevalence of MMC in Sweden might be as high as 8.0/10 000 births. This prevalence is one of the highest in Europe and comparable with that of South America and Mexico.

Today, almost 10% of all Swedish pregnant women have prenatal chromosomal analysis performed. The prevalence of DS among new-born children has, however, not decreased from that during the last decades. The median maternal age has increased very much during the last 30 years (from 26.5 years 1973 to 30.6 years 2003). The prevalence of DS in infants is today about 13-14/10 000 births. Without prenatal chromosome diagnosis combined with ToP that prevalence of DS could have been 20/10 000 births.
In 1978, the world’s first “test-tube baby”, Louise Brown, was born in the United Kingdom. It has been estimated that between 13 and 24% of couples of reproductive age (according to data provided by the Fertility Spanish Society: http://www.sefertilidad.com/infobasica/infogeneral/breve.php) have fertility problems, and recent studies suggest that this frequency could increase in the next years due to the influence of multiple factors. Assisted reproductive technologies have enabled many infertile couples having offspring. Consequently, the number of infants born every year after applying such techniques has been growing along the last decades all over the world. However, there are already many studies questioning the harmlessness of those procedures and, meanwhile it is clear their association to multiple pregnancies and some related complications (chiefly, prematurity), there are not so clear and universal conclusions regarding the risk for congenital defects.

We have epidemiologically analyzed data of the ECEMC (Spanish Collaborative Study of Congenital Malformations) to test the hypothesis suggested in 2001 by Anteby et al. These authors found a high frequency of ocular anomalies in a cohort of 47 children born after in vitro fertilization (IVF), although they discussed the limitations of their study, as their sample was rather biased. Subsequently, Cruysberg et al. (2002) and Moll et al. (2003), supported that hypothesis through the publication of 1 and 5 cases with retinoblastoma, respectively.

In the ECEMC data, after excluding familial cases and those of known cause, whether chromosomal or environmental, we have observed a significant increase of the global risk for congenital major ocular defects (detected during the first 3 days of life), among infants born after IVF or ICSI (OR=4.91; 95%CI: 1.29-13.29; p=0.00007). Interaction between IVF and twinning could not be excluded and this was taken into account when applying diverse models of conditioned logistic regression, conditioning by year and place of birth, and controlling relevant potential confounders (twinning, maternal age, multiparity, previous spontaneous abortions). In our data, at least in singleton pregnancies, there is a significant increase of the risk, derived from IVF, for the mentioned defects (OR=5.21; 95%CI: 1.37-19.90; p=0.016). We could not control by ethnic group due to the sample size for nonwhite ethnic groups.

Therefore, we consider that it could be justified to include a thorough ophthalmological examination in the follow-up of babies born after IVF.

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Comparison of Specific Congenital Anomaly Rates between five countries

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This study compares Maltese congenital anomaly rates with other countries. The diverse risk factors for each specific anomaly group make the overall rates less informative. A comparison table has been compiled by grouping defects by ICD-10 codes.

Malta Congenital Anomalies Registry (MCAR) data for the years 1995-2001 was used. East Sicilian data, based on Indagine Siciliana Malformazioni Congenite (ISMAC) data (1991 to 1998), has been used due to geographical proximity. EUROCAT data (1990 to 1999) gives an overview of the European experience. As a proxy global average rate, national data from Australia (1981 to 1997) and New York State (1995 to 1997) was used, in view of the cosmopolitan population in these regions.

One notices that Malta has the highest rate of malformations, namely cardiovascular and, to a lesser extent, neural tube defects.

One bias of these rates is that, being a small population, Maltese rates may be over- or underestimated. Moreover, while care has been taken to ensure maximum similarity between category definitions among countries, a few differences still exist.

The unavailability of therapeutic abortion in Malta could explain the higher Maltese rates. However, Malta has lower Down’s syndrome rates. The higher cardiovascular anomaly rate could also be due to differences in registration criteria.
Apple Peel Intestinal Atresia in a Miscarried Fetus

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Introduction
We report on a variant of apple peel atresia in a 19 week female fetus in a triplet pregnancy, born to non-consanguineous parents, and spontaneously miscarried at 19 weeks gestation. Apple peel intestinal atresia derives its name from a distal loop of small intestine twisted around the marginal artery.

Findings
The mother was a 27-year-old lady who had a six-year-old, apparently healthy son. She had polycystic ovaries and secondary infertility, and had ovarian stimulation with clomiphene, which was followed by a triplet pregnancy. She was prescribed dydrogesterone and folic acid, and a low calorie diet because of mildly deranged blood glucose levels. At 19 weeks gestation, she miscarried all 3 fetuses.

Triplet 1 was a female foetus whose crown-rump length, occipito–frontal and biparietal diameters corresponded to dates. There were no obvious external abnormalities. Detailed post-mortem examination showed an abnormal arrangement of the intestines. The stomach was normal while the duodenum was grossly distended and meconium-stained, and the jejunum was short, distended with meconium and arranged in the form of a spiral with its apex anteriorly. The small intestine lacked a mesentery. There was a short atretic segment of small intestine extending to the ileo-caecal junction. The large intestine was collapsed; the caecum and appendix were situated in the midline just below the liver in contact with the ligamentum teres, while the rest of the large intestine was irregularly coiled on the left side of the abdomen. The anus was patent. The rest of the examination was normal.

Conclusions
Detailed pathological examinations of spontaneously miscarried fetuses are not performed routinely in most hospitals, although they may be of great practical importance in genetic counselling, in providing insights into the pathogenesis of anomalies and in detecting anomalies that are rarely encountered in neonates because of their early lethality.
Spatial Analysis of Orofacial Clefts Rates in Latin America to Disclose Teratogens

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Geographic and/or temporal differences in the birth defects rates and the frequency of risk factors are considered as the first approach in the study of birth defect causality. The aim of present work was to analyze the spatial distribution of cleft lip ± cleft palate (CLP) and cleft palate only (CP) in the different geographic regions sampled by the Latin American Collaborative Study of Congenital Malformations (ECLAMC). 5,045,033 hospital births, delivered in the 1967-2004 period in 258 hospitals of Latin America were included in the study. Information regarding the exact location of each hospital (latitude, longitude) was utilized for to calculate Euclidean distances among hospitals and the spatial grouping. The method of average linkage between groups was used to identify spatial clustering of 6038 cases of CLP and 2026 cases of CP. The CLP and CP rates (per 10,000 births) with their respective Poisson CI95% for each geographic group was compared with the expected rates according to ECLAMC: 11.96 (11.66-12.27) and 4.01 (3.84-4.19), respectively. Four geographical clusters presented CLP rates greater than that expected if cases were distributed randomly among geographic regions in Latin America (p<0.001): 1) North region of Chile, and La Paz and Cochabamba cities of Bolivia, 23.5 (20.82-26.53); 2) Southern region of Chile and Southwest region of the Patagonia-Argentina, 17.23 (14.58-20.22); 3) Mountain region of Ecuador, 15.46 (13.77-17.31); and 4) Northwest region of Argentina, 15.18 (13.78-16.68). Added this, two regions showed CP rates greater than that expected: 1) Minas Gerais and Espíritu Santo counties of Southeast of Brazil, 8.42 (6.18-11.19); 2) Rio de Janeiro and Sao Paulo counties of Southeast of Brazil, 5.16 (4.60-5.78). The use of spatial statistics to analyze hospital monitoring data -such as ECLAMC-, which has a spatial component can be an efficient methodology to the study of teratogenic factors and birth defect causality.