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Maternal and paternal licit and illicit drug use, smoking and drinking and autism spectrum disorder

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Abstract *The aim of this study was to investigate the association between maternal and paternal licit and illicit drug use, smoking and drinking and autism spectrum disorder (ASD). We conducted a case-control study with children and adolescents diagnosed with ASD and neurotypical individuals. The data were collected using a semi-structured questionnaire administered during interviews with the children's mothers or guardians. The following variables were analyzed: child sex and age; maternal and parental age; use of medicines before and during pregnancy; classes of medicines used during pregnancy; maternal and paternal smoking; maternal and paternal drinking; maternal and paternal illicit drug use. The data were analyzed using logistic regression and crude and adjusted odds ratios (OR). After adjustment, the results showed an association between maternal use of antipyretics/pain killers during pregnancy (OR = 2.26; 95%CI 1.29-3.95; p < 0.040) and ASD. No association was found between maternal and paternal smoking, drinking and illicit drug use before and during pregnancy and ASD. The findings suggest that the development of ASD is influenced by environmental factors.*

Key words *Autistic disorder, Medicine use, Smoking, Alcohol-related disorders, Illicit drugs*

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Introduction

Autism Spectrum Disorder (ASD) is described by the Diagnostic and Statistical Manual of Mental Disorders (DSM-V)[1] as a developmental impairment that leads to impaired communication and social interaction characterized by restricted, repetitive patterns of behavior, interests or activities manifested before age 3 years with or without accompanying intellectual impairment.

The prevalence of ASD in the United States is 1 in 36 children and the disorder is four times more common among boys than in girls. While the number of diagnosed cases has increased among both adults and children, it is unclear whether this increase is related to the identification of new cases using diagnostic criteria, increased incidence of ASD or the use of different methodologies by studies on this topic[2].

Evidence suggests that the etiology of ASD is influenced by both genetic and environmental factors[3-6]. Data from a study with monozygotic twins show that although ASD is influenced by heritability, variation in severity of symptomatology is affected by environmental factors[7]. Furthermore, a molecular study discussed the importance of epigenetic mechanisms for neurodevelopment, suggesting they may be a causative factor for ASD[6], reinforcing evidence of the contribution of environmental factors to the etiology of the disorder. Environmental factors potentially associated with ASD include medicine use[4,8-14], smoking[3,6,15-19], drinking[20,21] and illicit drug use[15,22] during pregnancy.

The classes of medicines utilized during pregnancy associated with the development of ASD include antipyretics/painkillers[4,11,13], antidepressants[12], antiepileptic drugs[4,8,10] and antibiotics[9,13,14]. This association has been observed when exposure to the drug occurs in the first and second trimesters[14] or second and third trimesters[23] or throughout pregnancy[11]. However, other studies found no association between maternal drug utilization and ASD[24-27].

With regard to smoking, drinking and illicit drug use, the findings on exposure to smoking are inconclusive, with some studies reporting no significant association with ASD[28-30] and others finding that maternal and/or paternal smoking is related to a greater chance of ASD[3,6,15-19]. Studies on the relationship between drinking[20,21] and illicit drug use[15,22] during pregnancy are scarce and findings are inconsistent.

Although studies of paternal licit and illicit drug use, smoking and drinking are also scarce, the findings suggest that paternal smoking may influence the development of ASD, especially due to the effects that chemicals in cigarettes have on sperm production[3,30].

The present study is justified by the lack of information on this topic in the state of Minas Gerais and is the first survey of ASD in the state of this size (248 cases and 886 controls) investigating different variables (licit and illicit drug use, smoking and drinking) and including different classes of medicines. The aim of this study was to investigate the association between maternal and paternal licit and illicit drug use, smoking and drinking and ASD among children and adolescents in Minas Gerais, Brazil.

Methodology

We conducted an epidemiological case-control study to investigate the association between maternal and paternal licit and illicit drug use, smoking and drinking and ASD among children and adolescents in Montes Claros, Minas Gerais, Brazil.

The sample size was calculated based on the following parameters adopted for independent case-control studies: expected odds ratio (OR) of 1.9, probability of exposure in controls of 0.18, 80% statistical power, a significance level of 0.05 and four controls per case. We adopted a sample design effect (*deff*) of 1.5 and the sample was increased by 10% to account for potential losses. The resulting minimum necessary sample size was 213 cases and 930 controls.

The cases were selected from eight clinics that provide services for patients diagnosed with ASD and the *Associação Norte-mineira de Apoio ao Autista* (the north of Minas autism support association - ANDA). The inclusion criteria were patients with an ASD diagnosis report whose parent or guardian replied yes to the following question: "Has your child been diagnosed with ASD?" The controls were selected from the 63 public, private and philanthropic schools attended by the cases. The inclusion criteria were neurotypical children and adolescents without the characteristic features of ASD in the same age groups as the cases whose parent or guardian replied no to the following question: "Has your child been diagnosed with ASD?" Individuals who presented signs of ASD after screening using the Modified Checklist for Autism in Toddlers (M-CHAT) were excluded from the control group. Individuals who had comorbidities associated with ASD, such

as Down, Ret and fragile-X syndrome, were excluded from both groups. We set a ratio of four controls to each case and sought to select equal proportions of cases and controls across age groups. Further details about case and control group composition and sampling can be found in a previous study[31].

Data were collected using a 213-item semi-structured questionnaire prepared after conducting a literature review and revised by a multiprofessional team. The items were subdivided into eight groups: participant characteristics, parental demographic and socioeconomic characteristics, antenatal/neonatal/postnatal and family factors and birth events. The questionnaires were prescheduled and held in a location agreed previously with the child's mother/guardian. The interviews were scheduled and administered by a team of medical and nursing students taking part in a science internship program. The team received prior training to standardize procedures. Data collection was preceded by a pilot study to identify and correct any flaws in the tool.

The independent variables were paternal and maternal age, child sex, medicine use before and during pregnancy, classes of medicines used by the mother during pregnancy (antipyretics/painkillers, corticosteroids, antibiotics, anticonvulsants, antidepressants, anti-inflammatory drugs, hormones, antiemetics and other classes of medicines), maternal and paternal smoking, maternal and paternal drinking, and maternal and paternal illicit drug use. Maternal and paternal smoking, drinking and illicit drug use were defined as any type of use before and/or during pregnancy regardless of quantity. The variable other classes of medicines refers to medicines used during pregnancy mentioned by the mother that were not specified in the questionnaire. The association between the independent variables and ASD was assessed using the Mann-Whitney test for numerical variables and chi-squared test ($\chi 2$) for categorical variables.

Variables with a p-value of less than 0.20 in the bivariate analysis were included in the multivariate analysis, which was performed using a backward stepwise logistic regression model and adopting a significance level of 0.05. The strength of the association between the independent variables and outcome (case-control) was measured using crude and adjusted odds ratios (OR) and their respective 95% confidence intervals. Three models were run: Model 1, including maternal medicine use before and during pregnancy; Model 2, including medicine use before pregnancy and during specific pregnancy stages (only in the first trimester, in other trimesters and didn't use medicines during pregnancy); and Model 3, in which, in addition to medicine use before pregnancy, we included the variable class of medicine used (antipyretics/pain killers, antibiotics, other medicines, didn't use medicines). All models were adjusted according to child sex and mother's and father's age on the date the child was born. Goodness of fit was measured using the Hosmer-Lemeshow test. Statistical analyses were performed using SPSS version 23.0.

The study was conducted in accordance with the norms and standards set out in National Health Council Resolution 466/2012 regulating research involving human beings and approved by the research ethics committee (reference nº 534.000/14). The mothers who agreed to participate in the study signed an informed consent form after having the purpose and procedures of the study explained to them.

Results

The case and control groups comprised 248 and 886 children and adolescents, respectively, totaling 1134 participants. The case-control ratio was 1 to 3.6.

The mean age of the case and control groups was practically the same ($p = 0.521$; 6.4 years, ± 3.6 and 6.6 years, ± 3.4, respectively). The ages of the two groups were similar, with 43.6% of the overall sample being aged 2-5 years, 42.0% aged 6-10 years and 14. 4% aged over 10 years ($p = 0.318$).

In the bivariate analysis, 50.8% of the mothers from the case group and 42.2% from the control group were aged 30 years and over ($p = 0.016$) on the date the child was born, while 38.7% of fathers from the case group and 32.8% from the control group were aged over 35 years ($p = 0.085$).

Eighty-one per cent of the case group and 50.7% of the control group were male and 19% of the case group and 49.3% control group were female. A positive association was observed between being male and ASD (crude odds ratio = 4.16), showing that cases were more likely to be male (p-value < 0.001).

Thirty-five per cent of the mothers from the case group and 24.7% of the mothers in the control group reported using medicines before pregnancy. These percentages were 50% and 35.3%, respectively, for medicine use during pregnancy.

The results of the crude analysis revealed a significant positive association between medicine use before and during pregnancy and ASD (OR = 1.64; CI = 1.21-2.22 and OR = 1.83; CI = 1.38-2.44, respectively). The strength of this association was higher for medicine use during pregnancy (Table 1).

The results of the bivariate analysis also showed a positive association between medicine use only in the first trimester and in other trimesters and ASD (OR = 2.11; CI = 1.41-3.16 and OR = 1.70; CI = 1.22-2.73, respectively) (Table 1); however, the data also show that the women reported medicine use in the first trimester, second trimester, third trimester, in two trimesters or in all trimesters. The classes of medicines that showed a significant association with ASD were antipyretics/painkillers (OR = 2.48; CI = 1.52-4.06) and antibiotics (OR = 1.98; CI = 1.15-3.43) (Table 1), with 24.6% of women from the case group and 8.6% from the control group confirming that they used medicines not investigated by this study, including blood pressure medications, anticoagulants and antidiabetics (Figure 1).

No significant association was found between maternal and paternal smoking before (OR = 1.27; CI = 0.82-1.98 and OR = 1.03; CI = 0.74-1.41, respectively) and during pregnancy (OR = 0.85; CI = 0.34-2.09 and OR= 0.86; CI = 0.56-1.30, respectively) (Table 2).

The percentage of women who reported drinking during pregnancy was the same in each group (6.6% and 6.6%). There was no association between this variable and ASD (OR = 1.01; CI = 0.57-1.79). Most men (61.5% from the case group and 57.2% from the control group) reported drinking before pregnancy. The percentage of men who drank during pregnancy was higher than that of women, with similar rates being found in each group (48.7% in the case group and 48% in the control group). No association was found between paternal drinking during pregnancy (OR = 1.03; CI = 0.77-1.37) and the outcome (Table 2).

Illicit drug use was more frequent among men (3.5% in the case group and 3.1% in the control group) than in women (1.2% in the case group and 1.9% in the control group). No association was found between maternal illicit drug use before and during pregnancy (OR = 0.65; CI = 0.19-2.25) and paternal use before pregnancy (OR = 1.14; CI = 0.51-2.55) and ASD (Table 3).

In the multiple regression analysis adjusted for child sex and maternal and paternal age (Model 1), the positive association between medicine use before and during pregnancy (OR = 1.44; CI = 1.05-1.98 and OR = 1.71; CI = 1.27-2.30, respectively) and ASD remained significant (Table 4). In Model 2 there was a significant positive association between medicine use before pregnancy (OR = 1.43; CI = 1.02-1.99) and ASD and using medicine only in the first trimester and in other semesters increased the likelihood of ASD by 1.93 and 1.43 times, respectively (CI = 1.25-2.96 and CI = 1.01-2.05). The results of Model 3 also showed a significant association between medicine use before pregnancy and ASD (OR = 1.41; CI = 1.01-1.96). A significant positive association was found between the use of antipyretics/pain killers (OR = 2.26; CI = 1.29-3.95) and other classes of medicines (OR = 1.46, CI = 1.04-2.12) during pregnancy and ASD. However, the association between antibiotics use and ASD was borderline in this model. Finally, when the models were tested it was observed that the association was related to medicine type rather than the number of medicines used during pregnancy.

Discussion

Our findings show an association between maternal use of antipyretics/painkillers and antibiotics before and anytime during pregnancy and the outcome. No association was found between maternal and paternal smoking, drinking and illicit drug use before and during pregnancy and ASD.

The results were similar between the case and control groups when broken down by child age. The findings show that children and adolescents from the case group were more likely to have a mother aged over 30 years and father under 35 years, corroborating the results of other studies[13,24,32].

The association between maternal medicine use before and during pregnancy and ASD has been confirmed by other studies[4,8-14]. This is explained by the fact that these substances cross the placental and blood–brain barriers, affecting the central nervous system of the growing fetus, resulting in newborn complications such as cognitive impairment, malformations and neonatal abstinence syndrome[11].

Our findings show that the strength of association between using medicines only in the first trimester and ASD was higher than for use in other trimesters. There is a divergence between the findings in the literature on this matter, with data suggesting increased risk of ASD particularly for

Table 1. Medicine use before and during pregnancy and classes of medicines by case and control group. Crude odds ratios with respective confidence intervals. Montes Claros (Brazil).

Variables	Case (n = 248)	Control (n = 886)	Total (1,134)	$_{crude}$OR (95%CI)	p-value*
	n(%)	n(%)	n(%)		
Medicine use					
Used medicine (before pregnancy)					
Yes	86 (35.0)	209 (24.7)	295 (27.0)	1.64 (1.21-2.22)	0.001
No	160 (65.0)	637 (75.3)	797 (73.0)	1.00	
Used medicine (during pregnancy)					
Yes	123 (50.0)	310 (35.3)	433 (38.5)	1.83 (1.38-2.44)	< 0.001
No	123 (50.0)	568 (64.7)	691 (61.5)	1.00	
Pregnancy stage when medicine was used					
Other semesters	73 (30.3)	197 (22.9)	270 (24.5)	1.70 (1.22-2.37)	< 0.001
Only in the first semester	45 (18.7)	98 (11.4)	143 (13)	2.11 (1.41-3.16)	
Didn't use medicine	123 (51)	565 (65.7)	688 (62.5)	1.00	
Classes of medicines used by the mother during pregnancy					
Antipyretics/painkillers					
Yes	23 (9.7)	46 (5.4)	69 (6.3)	2.48 (1.52-4.06)	< 0.001
Other	91 (38.4)	242 (28.3)	333 (30.5)	1.74 (1.28-2.37)	< 0.001
No	123 (51.9)	568 (66.3)	691 (63.2)	1.00	
Corticosteroids					
Yes	4 (1.6)	7 (0.8)	11 (1.0)	2.64 (0.76-9.17)	0.126
Other	116 (47.7)	289 (33.4)	405 (35.7)	1.86 (1.39-2.48)	< 0.001
No	123 (50.6)	569 (65.8)	692 (62.5)	1.00	
Antibiotics					
Yes	21 (8.6)	49 (5.7)	70 (6.3)	1.98 (1.15-3.43)	0.014
Other	99 (40.7)	247 (28.6)	346 (31.3)	1.85 (1.37-2.51)	< 0.001
No	123 (50.6)	568 (65.7)	691 (62.4)	1.00	
Anticonvulsants					
Yes	3 (1.2)	7 (0.8)	10 (0.9)	1.98 (0.51-7.78)	0.326
Other	117 (48.1)	189 (33.4)	406 (36.6)	1.87 (1.40-2.50)	< 0.001
No	123 (50.6)	569 (65.8)	692 (62.5)	1.00	
Antidepressants					
Yes	4 (1.6)	5 (0.6)	9 (0.8)	3.70 (0.98-13.98)	0.054
Other	116 (47.7)	291 (33.6)	407 (36.7)	1.84 (1.38-2.47)	< 0.001
No	123 (50.6)	569 (65.8)	692 (62.5)	1.00	
Anti-inflammatory drugs					
Yes	3 (1.2)	13 (1.5)	16 (1.4)	1.07 (0.30-3.80)	0.920
Other	117 (48.1)	283 (32.7)	400 (36.1)	1.91 (1.43-2.56)	< 0.001
No	123 (50.6)	569 (65.8)	692 (62.5)	1.00	
Hormones					
Yes	10 (4.1)	22 (2.5)	32 (2.9)	2.10 (0.97-4.55)	0.059
Other	110 (45.3)	274 (31.7)	384 (34.7)	1.86 (1.38-2.49)	< 0.001
No	123 (50.6)	569 (65.8)	692 (62.5)	1.00	
Antiemetics					
Yes	27 (11.1)	125 (14.5)	152 (13.7)	1.00 (0.63-1.58)	0.997
Other	93 (38.3)	171 (19.8)	264 (23.8)	2.52 (1.83-3.46)	< 0.001
No	123 (50.6)	569 (65.8)	692 (62.5)	1.00	

OR = odds ratio; *chi-squared test.

Source: Authors.

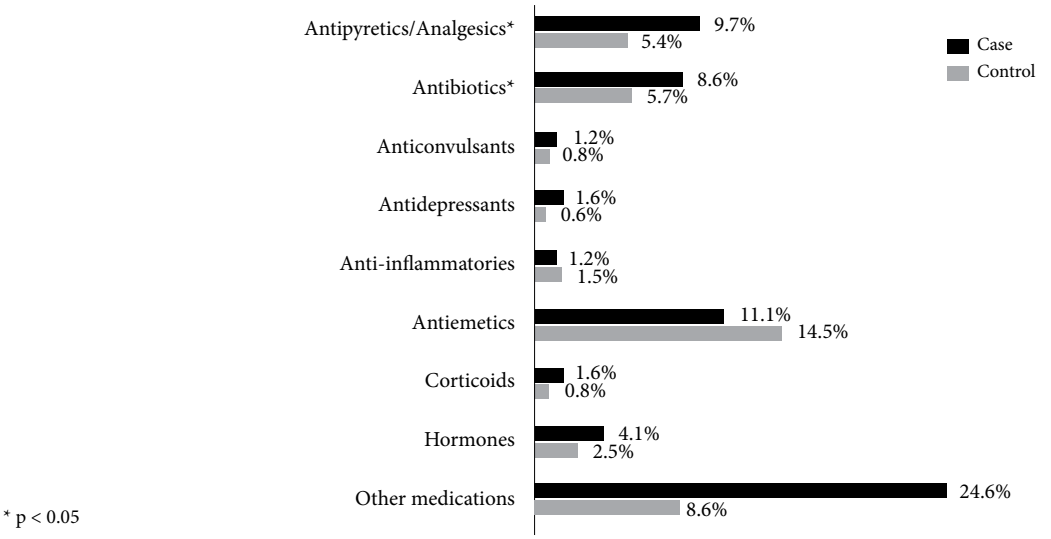

Figure 1. Classes of medicines used during pregnancy. Percentage use in the case and control groups.

Source: Authors.

Table 2. Maternal and paternal smoking and drinking by case and control group. Crude odds ratios with respective confidence intervals. Montes Claros (Brazil).

Variables	Case (n = 248)	Control (n = 886)	Total (1.134)	$_{crude}$OR (95%CI)	p-value*
	n(%)	n(%)	n(%)		
Maternal smoking					
Are you or have you ever been a smoker?					
Yes	30 (12.1)	85 (9.7)	115 (10.3)	1.27 (0.82-1.98)	0.282
No	218 (87.9)	787 (90.3)	1005 (89.7)	1.00	
Did you smoke during pregnancy?					
Yes	6 (2.4)	25 (2.9)	31 (2.8)	0.85 (0.34-2.09)	0.721
No	239 (97.6)	845 (97.1)	1084 (97.2)	1.00	
Paternal smoking					
Are you or have you ever been a smoker?					
Yes	66 (26.9)	228 (26.5)	294 (26.6)	1.03 (0.74-1.41)	0.879
No	179 (73.1)	634 (73.5)	813 (73.4)	1.00	
Did you smoke during pregnancy?					
Yes	31 (12.7)	125 (14.6)	156 (14.1)	0.86 (0.56-1.30)	0.534
No	213 (87.3)	734 (85.4)	947 (85.9)	1.00	
Maternal drinking					
Drinking (during pregnancy)					
Yes	16 (6.6)	57 (6.6)	73 (6.6)	1.01 (0.57-1.79)	0.984
No	226 (93.4)	810 (93.4)	1036 (93.4)	1.00	
Paternal drinking					
Drinking (before pregnancy)					
Yes	147 (61.5)	483 (57.2)	630 (58.2)	1.14 (0.89-1.60)	0.236
No	92 (38.5)	361 (42.8)	453 (41.8)	1.00	
Drinking (during pregnancy)					
Yes	113 (48.7)	405 (48.0)	518 (48.2)	1.03 (0.77-1.37)	0.858
No	119 (51.3)	438 (52.0)	557 (51.8)	1.00	

OR = odds ratio; *chi-squared test.

Source: Authors.

Table 3. Maternal and paternal use of illicit drugs by case and control group. Crude odds ratios with respective confidence intervals. Montes Claros (Brazil).

Variables	Case (n = 248)	Control (n = 886)	Total (1,134)	$_{crude}$OR (95%CI)	p-value*
	n(%)	n(%)	n(%)		
Maternal illicit drug use					
Has used or uses drugs					
Yes	3 (1.2)	16 (1.9)	19 (1.7)	0.65 (0.19-2.25)	0.493
No	244 (98.8)	846 (98.1)	1090 (98.3)	1.00	
Paternal illicit drug use					
Used drugs before pregnancy					
Yes	8 (3.5)	26 (3.1)	34 (3.2)	1.14 (0.51-2.55)	0.752
No	220 (96.5)	814 (96.9)	1034 (96.8)	1.00	

OR = odds ratio; *chi-squared test.
Source: Authors.

Table 4. Multiple regression model of maternal medicine use before and during pregnancy associated with autism spectrum disorder. Adjusted odds ratios with respective confidence intervals. Montes Claros (Brazil).

Variables	Model 1* (n = 1,085)		Model 2** (n = 1,064)		Model 3*** (n = 1,071)	
	adjOR ($CI_{95\%}$)	p-value	adjOR ($CI_{95\%}$)	p-value	adjOR ($CI_{95\%}$)	p-value
Used medicine (before pregnancy)						
Yes	1.44(1.05-1.98)	0.023	1.43 (1.02-1.99)	0.014	1. 41 (1.01-1.96)	0.045
No	1.00		1.00		1.00	
Used medicine (during pregnancy)						
Yes	1.71(1.27-2.30)	< 0.001				
No	1.00					
Pregnancy stage when medicine was used						
Other semesters			1.43 (1.01-2.05)	0.049		
Only in the first semester			1.93 (1.25-2.96)	0.003		
Didn't use medicine			1.00			
Class of medicine used during pregnancy						
Antipyretics/pain killers					2.26 (1.29-3.95)	0.040
Antibiotics					1.63 (0.92-2.90)	0.095
Other classes of medicines					1.49 (1.04-2.12)	0.022
Didn't use medicine					1.00	

adjOR = odds ratio adjusted for child sex and maternal and paternal age. HL test (p = 0.613). HL test (p = 0.753). HL test (p = 0.957).

Source: Authors.

medicine use in the first and second trimesters, which is when the initial phases of neurodevelopment take place, such as cell division and neuronal growth and migration[14]. In contrast, other studies have reported increased risk of autism in children whose mothers used medicines during the second and/or third trimesters, suggesting a relationship with DNA methylation[8,9]. Another study also found an association between medicine use anytime during pregnancy and ASD[11].

In the present study, the classes of medicines associated with the development of ASD were antipyretics/painkillers and antibiotics. The most commonly cited antipyretic/painkiller in the literature was paracetamol (acetaminophen), with some studies showing an association between this drug and ASD[4,11,13]. Prenatal exposure to paracetamol can increase the risk of autism by 20%, particularly when exposure is prolonged (20-28 days)[4,11]. Taking acetaminophen during

pregnancy can trigger maternal immune activation, which can effect fetal brain development, impair neurogenesis and cause oxidative stress[4,11]. It is estimated that around 50% of women use paracetamol during pregnancy, with the use of the drug being reported in the first and second trimesters by 69.9% of US women and 57.6% of Brazilian women[23].

Other studies have also reported a positive association between the use of antibiotics and ASD, pointing to increased risk of autism with the use of beta-lactam antibiotics and penicillin, particularly when exposure is longer than 15 days. No gender differences were observed[9,13,14]. The association between antibiotic use and ASD differs according to pregnancy stage (first and second trimesters and second and third trimesters)[23], suggesting that the development of autism may be related to the mother's ability to metabolize drugs at different stages of the pregancy[14]. Antibiotics can induce a specific mutation that explains the association with ASD; however, the use of these drugs also reflects the severity of infections during pregnancy, which can also be related to autism[11,13]. The results of studies with mice showed that antibiotics induced changes in fetal microbiota composition, which can disrupt the gut-brain axis, potentially impairing neurodevelopment and increasing the risk of ASD[9].

The literature also shows an association between the use of other classes of medicines such as antidepressants[12] and antiepileptic drugs[4,8,10] and ASD, and suggests that the use of recommended doses of folic acid, omega 3 and vitamin D can act as a protective factor against autismo[32-34]. The results of the present study are borderline when it comes to the association between the use of antidepressants and hormones and ASD. Just one systematic literature review showed increased risk of ASD in children whose mothers took antidepressants in the second and third trimesters of pregnancy[12]. Other studies show that the association between use of antidepressants during pregnancy and ASD loses its significance when confounding factors, particularly maternal depression and genetic predisposition, are adequately controlled[25-27,35]. One study showed that the use of antiepileptic drugs in the second and third trimesters at higher daily doses (more than 100 mg), particularly topiramate and valproate, may be related to increased risk of ASD[8].

Our data did not reveal an association between maternal and paternal smoking before and during pregnancy and ASD and the findings in the literature on this topic are inconclusive on this factor. Some studies found that both maternal and paternal smoking during pregnancy was associated with increased risk of autism[15,16]. Direct fetal exposure to chemicals in cigarette smoke can have effects on sperm production or the placenta, leading to oxygen deprivation and causing dysregulation of nicotinic receptors and affecting immune development, mechanisms which are potentially relevant to autism development[3,6,17]. Active or passive maternal smoking anytime during pregnancy has also been related to increased risk of ASD, particularly when it involves more than 20 cigarettes a day[6,16,17]. In contrast, other studies assessing serum cotinine levels (a nicotine biomarker) during pregnancy among mothers who smoke or are exposed to tobacco smoke found no association between maternal smoking and ASD[28,29].

The relationship between paternal smoking and ASD has not been systematically studied. The few studies found on this topic produced divergent results, reporting that this factor does not contribute to the development of autism[30] or demonstrating that prenatal paternal smoking is associated with a modest increase in risk of ASD, even after adjusting for potential confounding factors such as maternal smoking and drinking, socioeconomic status and family history of psychiatric disorders, which is an indicator of genetic risk of autism[3,16,18,19]. Studies agree that smoking is a common modifiable risk factor and a public health problem that has several impacts on human well-being, emphasizing the need for further research on sperm quality, germline mutations and epigenetic changes in sperm, which can have considerable inter and transgenerational impacts[19,30].

No level of alcohol is safe to drink during pregnancy; however, despite public health efforts, it is estimated that 10% of women worldwide continue to consume alcohol during pregnancy[36]. Alcohol can cross the placenta and accumulate in the amniotic fluid, having a prolonged effect on the fetus and being especially harmful to the development of the nervous system[36]. The present study did not find an association between maternal and paternal drinking before or during pregnancy and ASD, corroborating studies that found no association between light, moderate or heavy drinking during pregnancy and autism[20,21]. Studies investigating the relationship between maternal and paternal drinking during pregnancy and ASD are scarce. Data show that prenatal drinking is associated with poorer child mental health; however, the limited sample size

of the studies means that it is not possible to draw clear conclusions about the relationship between drinking and autism[3,15,37]. It is worth highlighting the differences in the percentage of women and men who drank during pregnancy in both the case and control groups in the present study.

Our findings show that illicit drug use was more frequent among men than women in both groups; however, this study found no association between maternal and paternal drug use and ASD. Studies on this topic address maternal illicit drug use during pregnancy but do not discuss the effects of paternal drug use on fetal health. With the legalization of recreational cannabis, the use of drugs during pregnancy has increased, leading to concern about the potentially adverse childhood effects related to prenatal exposure[22]. The limited number of studies investigating the influence of the use of illicit substances during pregnancy on ASD among children suggest an association between maternal cannabis use and symptoms of autism, even after controlling for factors such as family income and the use of other substances, such as alcohol, cocaine, hallucinogens and opioids[15,22]. It is suggested that in utero drug exposure can lead to region- and gene-specific neural changes and defects in neuronal wiring due to the disruption of endocannabinoid signaling, affecting fetal development and increasing the risk of autism diagnosis by around 50%[22]. As with drinking, levels of maternal use of illicit drugs were low compared to those in men. We did not find any studies investigating the relationship between maternal and paternal illicit drug use before pregnancy and ASD.

The positive association between medicine use before and during pregnancy and ASD remained significant in Model 1 of the multiple regression analysis (Table 4). Other studies in which medicine use was also adjusted for child sex and maternal and paternal age also found an association between these variables and ASD[8,9].

In Model 2, the positive association between medicine use before pregnancy, only in the first trimester and in other trimesters and ASD remained significant. These findings are corroborated by the literature, with studies reporting a positive association when medicine use was in the first and second trimesters[14] or second and third trimesters[23].

The positive association between medicine use before and during pregnancy and between the use of antipyretic/pain killers and other classes of medicines and ASD remained unchanged in Model 3. The association between antibiotics use during pregnancy and ASD was not maintained in this model. In studies involving other classes of medicines used during pregnancy, the association between antiepileptic drugs[8] and ASD remained unchanged in the multivariate analysis, while the association between antidepressants was not maintained[25,26]. Another study shows that the association between antibiotics and ASD remained unchanged[9] after adjustment. In the present study, however, the result was borderline.

It is important to highlight that many studies only show if there was an association between licit and illicit drug use, smoking and drinking during pregnancy and ASD without clarifying which physiological mechanism gives rise to the association. The literature on this topic in Brazil, particularly in the state of Minas Gerais, is scarce, limiting some points of discussion and indicates the need for further research on this theme.

Study limitations include: (1) drinking and illicit drug use may have been underestimated due to recall bias; (2) the fact that the diagnosis of ASD was performed by different teams means that it was not possible to verify the diagnostic criteria used; (3) lack of information about characteristic features of autism in parents/guardians; and (4) the abstract nature of the questions about maternal drinking and illicit drug use before pregnancy.

Strengths include: the use of a list of potential confounding factors identified in the literature; the fact that the questionnaire was applied by a previously trained team; the high case-control ratio (almost 1 to 4); the use of random sampling to select controls who are representative of the general population; and screening of the children for signs of ASD. It is worth highlighting that this is the first study investigating ASD of this size in Minas Gerais.

Conclusion

The findings show an association between maternal medicine use before pregnancy and during the first semester or other semesters of pregnancy, particularly the use of antipyretics/painkillers and antibiotics, and ASD. However, the association between antibiotics use and ASD was not maintained after adjusting for child sex and maternal and paternal age. Since these classes of medicines are normally used for inflammations and/or infections, further research is required to investigate the possible influence of related mechanisms such as maternal immune activa-

tion on the association with autism. No association was found between smoking, drinking and illicit drug use and ASD. Our findings suggest that the development of ASD is influenced by environmental factors, drawing attention to the need to consider the potential effects of prenatal exposure to medicines. It is hoped that this study can contribute to future research on the screening, diagnosis, treatment and, more especially, the prevention of autism.

Collaborations

MTC Almeida: conception and design of the study, data collection, critical review and approval of the final version of the article. FA Maia: conception and design of the study, data collection, critical review and approval of the final version of the article. LF Rezende: critical review and approval of the final version of the article. VSA Saeger and SLN Oliveira: data collection, critical review and approval of the final version of the article. GL Mangabeira: critical review and approval of the final version of the article. MF Silveira: conception and design of the study, data collection, statistical analysis, critical review of the article, approval of the final version of the article.

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