Original Article

Paternal and maternal age at pregnancy and autism spectrum disorders in offspring

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Abstract

Background The prevalence of autism spectrum disorders (ASDs) has increased 10 times over the past half century, while paternal and maternal age at pregnancy has also increased. Studies looking for an association between paternal or maternal age at pregnancy and ASDs in offspring have not been conclusive.

Objective To assess for possible associations between paternal and maternal age at pregnancy and ASDs in offspring.

Methods This case-control study had 50 case and 100 control subjects, each case was matched for age and gender to two controls. Case subjects were obtained by consecutive sampling of patients aged 18 months to 7 years who visited the Developmental Behavioral & Community Pediatrics Outpatient Clinic and private growth and development centers from January to April 2013, while control group were children of the same age range and same gender who visited pediatric outpatient clinic at Sanglah Hospital mostly due to acute respiratory tract infection, without ASDs as assessed by the DSM-IV-TR criteria. We interviewed parents to collect the following data: maternal and paternal age at pregnancy, child's birth weight, history of asphyxia, hospital admission during the neonatal period, pathological labor, maternal smoking during pregnancy, paternal smoking, and gestational age. Data analysis was performed with Chi-square and Fisher's exact tests.

Results Multivariable analysis showed that higher paternal age at pregnancy was associated with ASDs in offspring (OR 6.3; 95%CI 2.0 to 19.3; P 0.001). However, there was no significant association between maternal age during pregnancy and the incidence of ASDs. Asphyxia and paternal smoking were also associated with higher incidence of ASDs in the offspring (OR 10.3; 95%CI 1.9 to 56.5; P 0.007 and OR 3.2; 95%CI 1.5 to 6.9; P 0.003, respectively).

Conclusion Paternal age ≥40 years increased the risk of ASDs in offspring by 6.3 times. In addition, paternal smoking increased the risk of ASDs in offspring by 3.2 times and asphyxia increased the risk of ASDs in offspring by 10.3 times. [**Paediatr Indones. 2015;55:345-51**].

Keywords: paternal age, maternal age, autism spectrum disorders

utism spectrum disorders (ASDs) are a group of pervasive developmental disorders characterized by impaired communication and social interaction, as well as restrictive and repetitive interests and behaviors.[1-3] Autism generally manifests within the first 3 years of life and is mostly diagnosed in children at about 3.5 years of age.[2] Autism prevalence was reported to be 1:303 and 1:94 in 14 places in the US, with an average 1:150 or 6.6 in every 1,000 children aged 8 years in 2007.[4] Indonesia does not have exact data on ASD prevalence, but the number of children

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diagnosed with ASDs is increasing.[5] The etiology of autism remains inconclusive, as both genetic and non-genetic factors are considered to play role in ASDs.[2,6-8]

The prevalence of ASDs has increased in recent decades. At the same time, average maternal and paternal ages at pregnancy also increased.[9] Durkin *et al.* reported that paternal and maternal ages were associated with ASDs in children.[10] In their study, maternal age >35 years and paternal age >40 years increased the risk of having children with ASDs. This increaesd risk of having children with ASDs may be due to spontaneous mutations and changes in genetic mapping that occur as a result of aging.[4,11,12] The aim of this study was to assess for possible relationships between paternal and maternal age at pregnancy and the incidence of ASDs in offspring.

Methods

This was a case-control study with age- and gender-matched subjects in the two groups, with or without ASDs. Each subjects in case group was matched with two subjects in control group. This study was conducted at the Developmental Behavioral & Community Pediatrics Outpatient Clinic in Sanglah Hospital and two private growth and development centers in Denpasar (*Pusat Tumbuh Kembang Anak Berkebutuhan Khusus Denpasar* and *Pusat Layanan Psikologi Pradnyagama Denpasar*) from January to April 2013. We interviewed parents to obtain data on maternal and paternal age at pregnancy, child's birth weight, history of asphyxia, hospital admission during the neonatal period, pathological labor, maternal smoking during pregnancy, paternal smoking, and gestational age. Informed consent was obtained from subjects' parents.

The inclusion criteria for the case group were children aged 18 months to 7 years, who were first diagnosed with ASDs at less than 3 years of age using the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, Text Revision (DSM-IV-TR) criteria.[13] Inclusion criteria for control group were children of the same age range and same gender who visited pediatric outpatient clinic at Sanglah Hospital mostly due to acute resp tract inf, without ASDs as assessed by the DSM-IV-TR criteria.

Exclusion criteria for both case and control groups were (1) major congenital abnormalities, e.g., Rett syndrome (diagnosed by DSM-IV-TR), Down syndrome (diagnosed by physical examination), tuberous sclerosis (by physical examination), neurofibromatosis (by physical examination) and fragile-X syndrome (by physical examination), (2) specific congenital infections, e.g., rubella, herpes simplex, syphilis, and varicella zoster, (3) those with deceased father or mother, as they could not be questioned, or (4) refusal to participate. Sample size was calculated by using a paired case-control formula and analysed for seven independent variables.[14] The independent variables were (1) paternal age at the mother's pregnancy and (2) maternal age at pregnancy. Confounding variables were (1) child's birth weight, (2) history of asphyxia, (3) history of hospital admission during the neonatal period, (4) pathological labor, (5) maternal smoking during pregnancy, (6) paternal smoking, and (7) gestational age.

Maternal age at pregnancy was defined to be the mother's age (in years) when her pregnancy was known for the first time, or on the first day of her last menstrual period. A maternal age of <35 years was considered to be optimal.[10] Paternal age at the time of the mother's pregnancy was defined to be the father's age at the time the mother became aware of her pregnancy. A paternal age of <40 years was considered to be optimal.[10] Child's birth weight was defined to be his weight at the first measurement after birth, and was considered to be abnormal for <2,500 grams or ≥4,000 grams.[15] History of hospital admission during the neonatal period was defined to be admission during the first month of life, due to any diagnosis. History of asphyxia was defined to be delayed crying by the neonate after delivery or the neonate undergoing intensive care with respiratory support after delivery. Pathological labor was defined to be delivery by either caesarean section, vacuum extraction, or forceps extraction. Maternal smoking was defined to be a history of smoking by the mother within a month of her pregnancy and during pregnancy, with at least one cigarette daily. Gestational age was defined to be the number of weeks of pregnancy at the time of birth, and was considered to be abnormal for <37 weeks or ≥42 weeks, as measured from first day of the last menstrual period.[15] Paternal smoking was defined to

be a history of the father smoking around the mother during her pregnancy.

We diagnosed children suspected to have ASDs and reassessed children with previously diagnosed ASDs before the age of three years who visited the Developmental Behavioral & Community Pediatrics Outpatient Clinic at Sanglah Hospital Denpasar and two private growth and developmental centers in Denpasar. At two private growth and developmental centers, we collected children who diagnosed as ASDs before age 3 years old by consultan in Developmental Behavioral & Community Pediatrics Outpatient Clinic at Sanglah Hospital Denpasar. We used ASDs criteria according to the DSM IV-TR to diagnosed and reassessed all the subjects.

Paired controls for case subjects were sought at the Pediatric Outpatient Clinic at Sanglah Hospital, Denpasar and referred to the Developmental Behavioral & Community Pediatrics Outpatient Clinic to rule out ASDs by the DSM-IV-TR criteria.

The association between variables was analyzed using Chi-square and Fisher's exact tests. The strength of the association between the independent and dependent variables was shown by odds ratio (OR), 95% confidence intervals (CI), and a level of significance of $P < 0.05$. Multivariate analysis with logistic regression was conducted on all independent variables. We performed the statistical analysis with *SPSS version 16* software.

This study was approved by the Research Ethics Committee of Udayana University Medical School, Sanglah Hospital, Denpasar.

Results

Fifty-two children with ASDs met the inclusion criteria. Of these, 1 child refused to participate and 1 child did not live with the biological parent, so that parent could not be questioned, resulting in 50 case
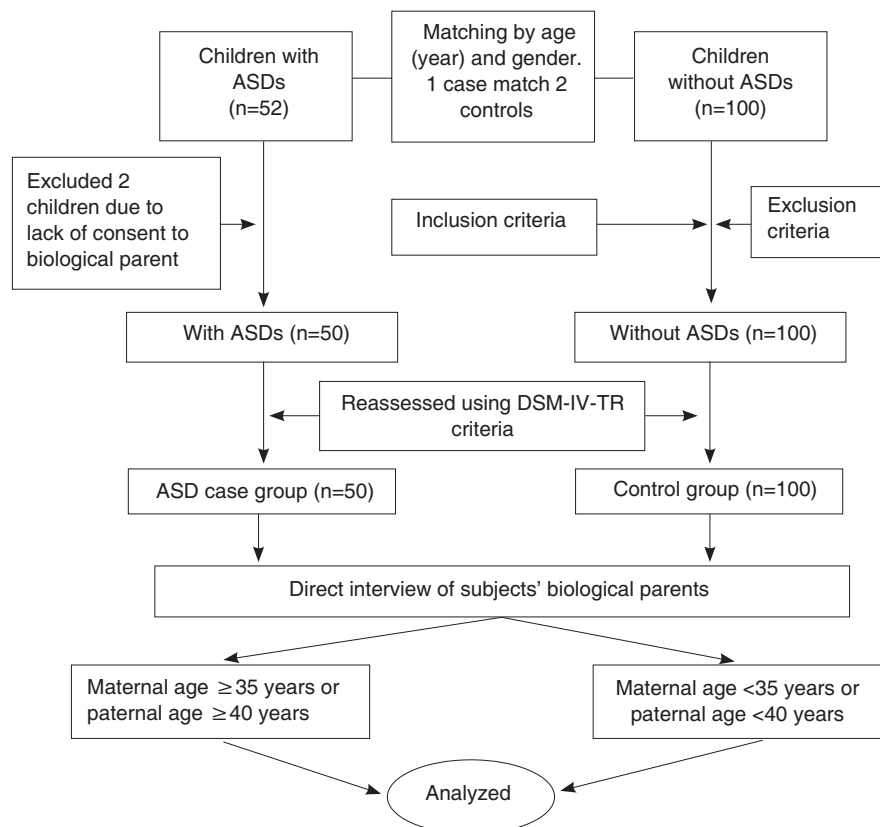

Figure 1. Study profile

subjects (**Figure 1**). This study sample consisted of 37 pairs of male subjects and 13 pairs of female subjects, each subjects was paired with two controls. Subjects' mean age was 4.56 (SD 1.37) years, ranging from 2 to 7 years. Subjects' characteristics are shown in **Table 1**.

Univariable analysis of both paternal and maternal age at pregnancy to ASDs in offspring

showed a statistically significant difference. **Table 2** show that significantly more older mother in the case group than in the control group (30% *vs*. 9%; P 0.027) and more older father in the case group than in the control group (22% *vs*. 6%; P 0.004)

The results of the bivariate analysis between confounding variables and ASDs in offspring are shown in **Table 3**. History of hospital admission during

Table 1. Subjects' characteristics

Characteristics	Case (n=50)	Control (n=100)
Male gender, n (%)	37 (74.0)	74 (74.0)
Mean age (SD), years	4.56 (1.37)	4.56 (1.37)
Abnormal birth weight, n (%)	11 (22.0)	20 (20.0)
Abnormal gestational age, n (%)	8 (16.0)	15 (15.0)
History of hospital admission during neonatal period, n (%)	8 (16.0)	6 (6.0)
History of asphyxia, n (%)	6 (12.0)	2 (2.0)
Maternal smoking, n (%)	1 (2.0)	0
Paternal smoking, n (%)	28 (56.0)	33 (33.0)

Table 2. Univariable analysis of paternal and maternal age to ASDs in offspring

Variables	Case (n=50)	Control (n=100)	P value	OR	95% CI
Paternal age at pregnancy, n (%)			0.004*	4.4	1.5 to 12.8
≥40 years	11 (22.0)	6 (6.0)			
<40 years	39 (78.0)	94 (94.0)			
Maternal age at pregnancy, n (%)			0.027*	2.9	1.1 to 7.4
≥35 years	15 (30.0)	9 (9.0)			
< 35 years	39 (70.0)	91 (91.0)			

*Chi-square test

Table 3. Univariable analysis between confounding variables and ASDs in offspring

Variables	Case (n=50)	Control (n=100)	P value	OR	95% CI
Birth weight, n (%)			0.78*	1.1	0.5 to 2.6
Abnormal	11 (22.0)	20 (20.0)			
Normal	39 (78.0)	80 (80.0)			
Gestational age, n (%)			0.87*	1.1	0.4 to 2.7
Abnormal	8 (16.0)	15 (15.0)			
Normal	42 (84.0)	85 (85.0)			
History of hospital admission during neonatal period, n (%)			0.02**	6.7	1.3 to 34.4
Yes	6 (12.0)	2 (2.0)			
No	44 (88.0)	98 (98.0)			
History of asphyxia, n (%)			0.07**	2.9	0.9 to 9.1
Yes	8 (16.0)	6 (6.0)			
No	42 (84.0)	94 (94.0)			
Maternal smoking, n (%)			0.33**	NA	NA
Yes	1 (2,0)	0			
No	0	0			
Paternal smoking, n (%)			0.007*	2.6	1.3 to 5.2
Yes	28 (56.0)	33 (33.0)			
No	22 (44.0)	67 (67.0)			

*Chi-square test; **Fisher's exact test; NA= not available

neonatal period and paternal smoking significantly higher in the case group than in the control group (12% *vs.* 2%; P 0.02 and 56% *vs.* 33%; P 0.007, respectively). Abnormal birth weight, abnormal gestational age, history of asphyxia, and maternal smoking were not significantly higher in the case group than in the control group (22% *vs* .20%; P 0.78, 16% *vs.* 15%; P 0.87, 16% *vs.* 6%; P 0.07, and 2% *vs.* 0; P 0.33, respectively)

All variables with P value <0.25 in bivariate analysis underwent multivariate analysis. A multivariate logistic regression with a backward stepwise method was used and the results are shown in **Table 4**. Paternal age at pregnancy was significantly correlated to the incidence of ASDs after adjustment for confounding variables (OR 6.3; 95%CI 2.0 to 19.3; P 0.001). However, maternal age was not associated with the occurrence of ASDs in offspring after adjustment for confounding variables. A history of asphyxia and paternal smoking were correlated with ASDs (OR 10.3; 95% CI 1.9 to 6.5; P 0.007 and OR 3.2; 95% CI 1.5 to 6.9; P 0.003, respectively) on multivariate analysis.

offspring by 6.3 times, compared to paternal age of <40 years. However, maternal age at pregnancy had no significant association with ASDs. Spontaneous mutations are more common in males than in females, since female germ cells undergo fewer cell divisions than male germ cells and stem cells accumulate mutations with each replication.[11]

Results from our study are consistent with an Israeli study which reported that advanced paternal age had a significant association with ASDs, both in bivariate and multivariate analyses after adjustment with confounding factors. They reported that paternal age >40 years increased the risk for ASDs in their offspring by 6 times, while maternal age had no significant association with ASDs in offspring.[17] Our study was consistent with a Japanese report that paternal age ≥33 years increased the risk for ASDs in their offspring by 3 times, while maternal age at pregnancy had no association with ASDs in offspring.[18]

In contrast, Croen *et al.* reported that the risk of autism significantly increased with each 10-year

Table 4. Logistic regression analysis between paternal and maternal age at pregnancy and the risk of ASDs in offspring, with adjustment for confounding variables

Variables	Step 1			Step 2			Step 3		
	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
Paternal age at pregnancy	4.5	1.2 to 16.6	0.024	4.4	1.2 to 15.9	0.026	6.3	2.0 to 19.3	0.001
History of asphyxia	5.2	0.5 to 57.1	0.177	10.9	1.9 to 60.3	0.006	10.3	1.9 to 56.5	0.007
Paternal smoking	3.4	1.6 to 7.5	0.002	3.3	1.5 to 7.0	0.003	3.2	1.5 to 6.9	0.003
Maternal age at pregnancy	2.0	0.6 to 6.9	0.261	1.9	0.6 TO 6.6	0.282			
History of hospital admission during neonatal period	2.2	0.4 to 13.7	0.394						

Discussion

Evidence for the etiology of ASDs remains inconclusive, with both genetic and environmental factors thought to play role in ASDs.[2] In recent decades, the prevalence of ASDs has increased at the same time that paternal and maternal age has increased. Advanced maternal and paternal age may increase the incidence of ASDs because of spontaneous mutations and/or changes in the genetic map the sperm or ovum.[10,16]

We found that paternal age ≥40 years at the time of pregnancy increased the risk for ASDs in their

increase in both maternal and paternal ages.[16] This inconsistency was probably caused by different methodologies. Their study was a historical birth cohort and analyzed paternal and maternal ages as continuous variables. When maternal and paternal age were analyzed as categorical variables, they reported that paternal age >34 years had a significant association with ASDs, but maternal age did not.

Furthermore, Durkin *et al.* reported maternal and paternal age to have significant associations with ASDs in offspring. They found that maternal age ≥35 years increased the risk for ASDs by 1.3 times in their

offspring, and paternal age ≥40 years increased the risk for ASDs by 1.4 times in their offspring. Their study was a cohort study using data from 10 participating study sites in the US Centers for Disease Control and Prevention's Autism and Developmental Disabilities Monitoring Network. There were 1,251 children aged 8 years with complete parental age information from the same birth cohort and identified as having an ASDs based on the DSM-IV-TR criteria. Parental and maternal ages were categorized as follows: <20, 20–24, 25–29, 30–34, 35–39, and >40 years.[10]

We found a significant association between asphyxia and the risk for ASDs in children. Oxygen requirement at birth is an indication of inadequate respiratory capability in newborns. Suitable oxygen delivery to all organs, including the brain, is critical. Asphyxia, at the extreme level, may cause irreparable damage to the brain resulting in abnormal brain development.[19] Our results were consistent with a Danish study which explored the association between perinatal factors, parental psychiatric history, socioeconomic status, and risk of autism. They reported a statistically significant association (even after adjustment for confounding factors) between asphyxia and the risk of autism in offspring.[20] In contrast, a Swedish study reported that a history of asphyxia was not significantly associated with the incidence of ASDs.[21] This difference was probably due to the data being sourced from the Swedish Medical Birth Register, which measured asphyxia history through Apgar scores, in contrast to our study in which history of asphyxia was assessed through interview.

Carbon monoxide and nicotine are the most dangerous components in cigarettes. They have toxic effects on the developing fetal nervous system.[22] Several studies reported an association between maternal smoking and the occurrence of autism in children, but none reported on a relationship between paternal smoking and autism. We found a significant association between paternal smoking at the time of pregnancy and the incidence of ASDs in offspring.

Our study had certain limitations. Our data may have been subject to recall bias, as it was collected by interview. We attempted to minimize this bias by restricting the age of the subjects included in this study. Cases and controls were recruited from different source populations, due to objections from case subjects' parents with regards to our visiting their neighborhood, and difficulties of finding controls from siblings. As such, control subjects were recruited from children who visited the Pediatric Outpatient Clinic at Sanglah Hospital, Denpasar. Another limitation of this study was the lack of cut-off point analyses between paternal and maternal age at pregnancy with the incidence of ASDs in offspring. Paternal age and maternal age were analyzed as categorical variables, and divided into two groups: paternal age at pregnancy of ≥40 years and <40 years, and maternal age at pregnancy of ≥35 years and <35 years, based on data from previous research.[10]

We observed a strong association between paternal age at pregnancy and ASDs in offspring. As such, we suggest that children with fathers ≥40 years of age at the time of pregnancy undergo early screening for ASDs. Further study with controls from the same population as the case subjects are needed to clarify the association between paternal age at pregnancy and ASDs in offspring. An analysis between paternal age and maternal age at pregnancy and the incidence of ASDs in children as continuous variables might be useful to obtain cut-off points for paternal and maternal age at pregnancy and ASDs. Further study is also required to confirm the association between paternal smoking and history of asphyxia and ASDs in offspring, as well as their potential interaction.

Acknowledgments

Conflict of interest

None declared.

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