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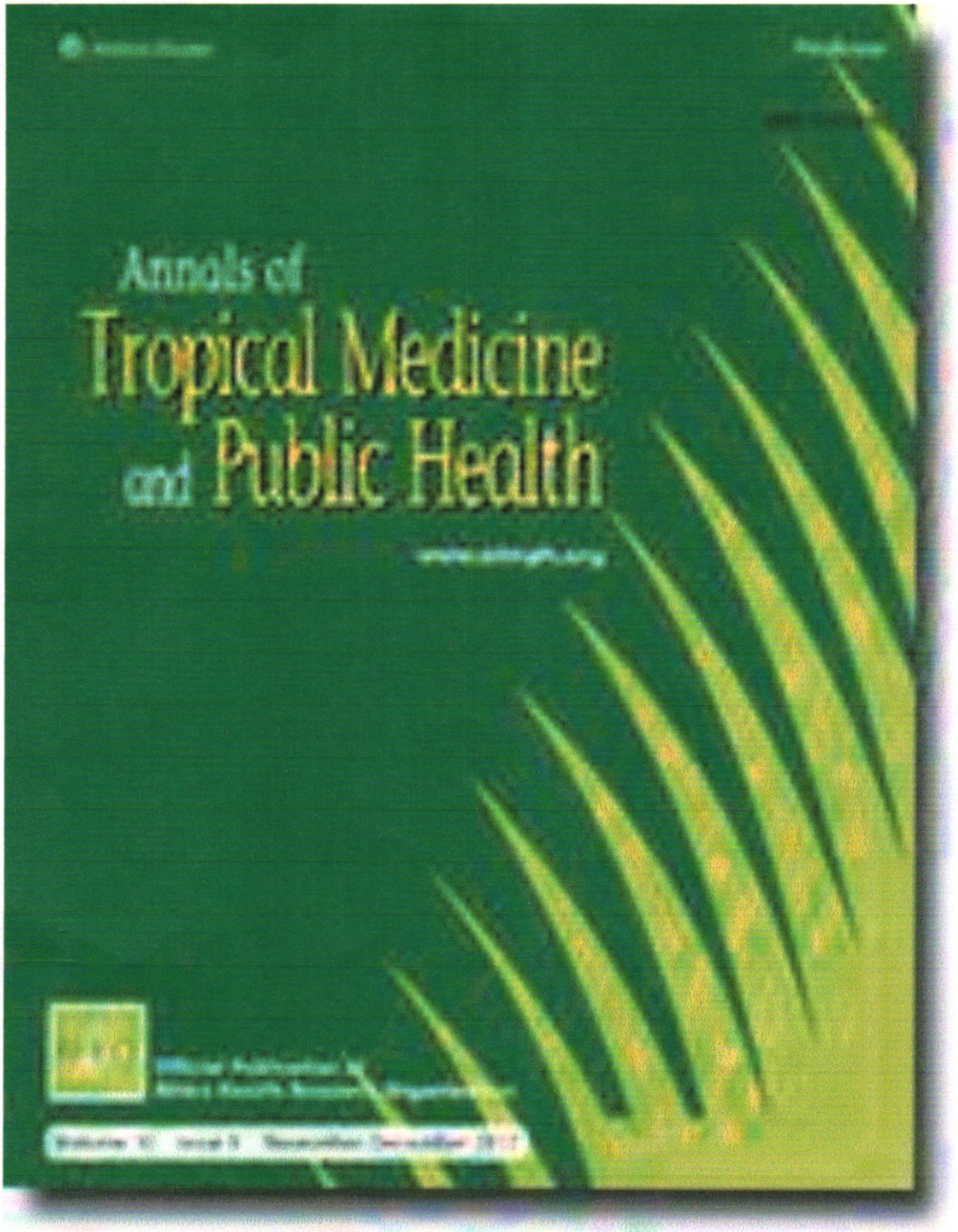

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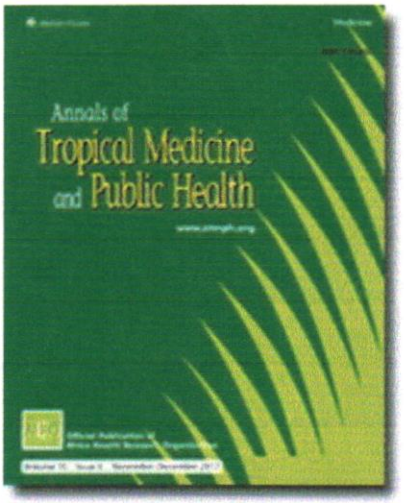

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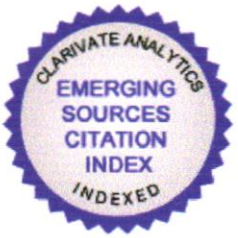

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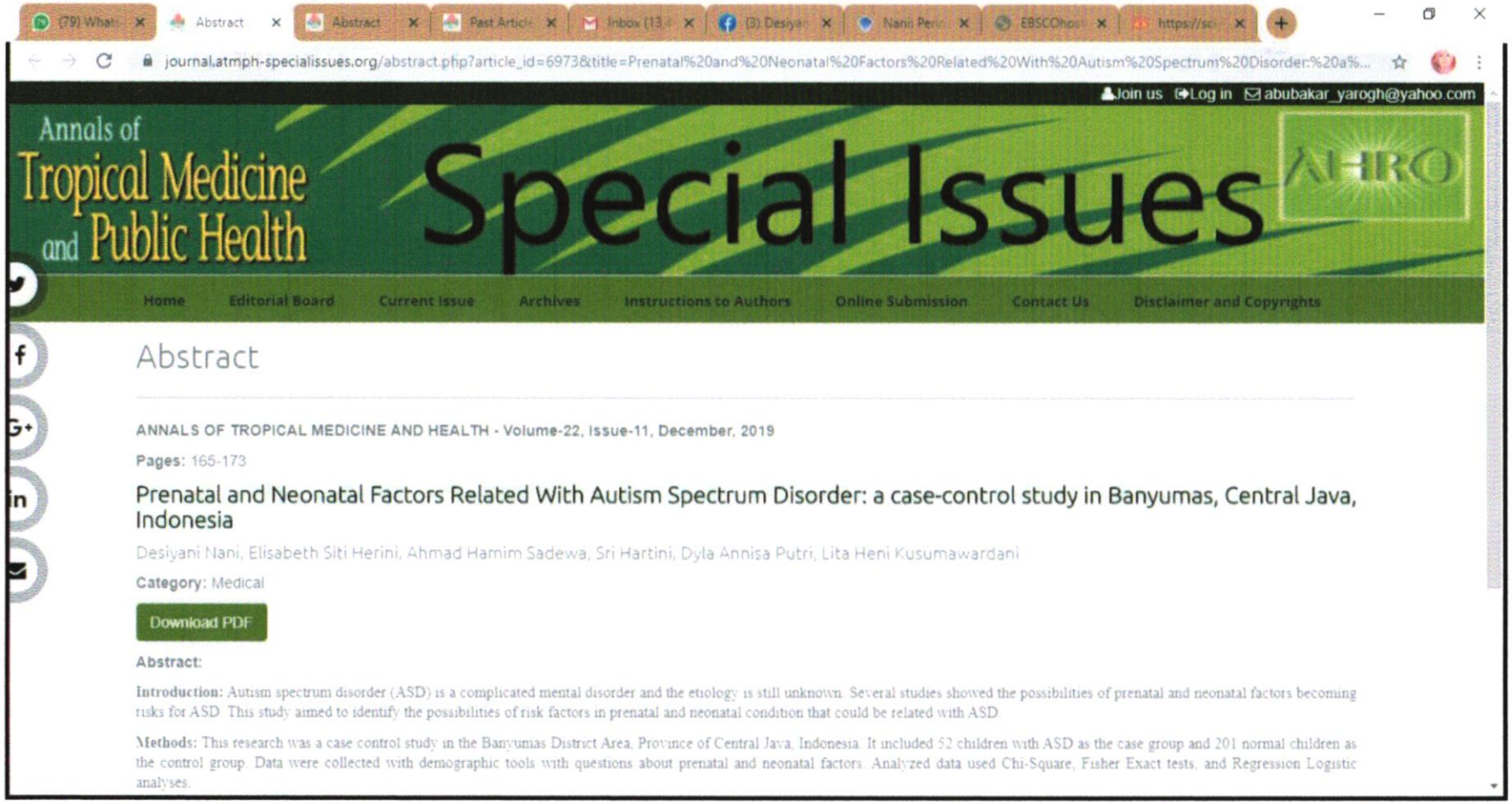

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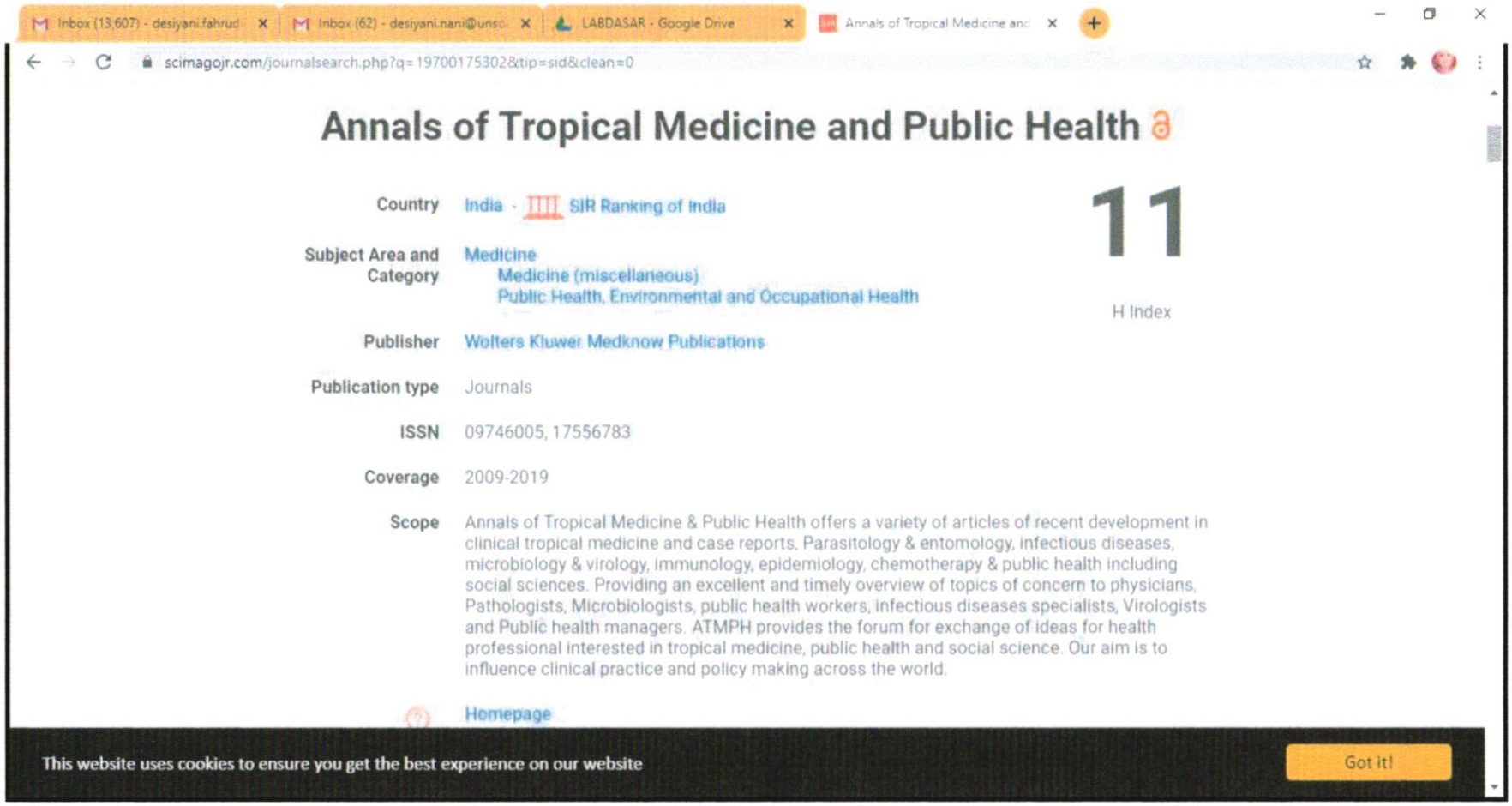

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STATEMENT LETTER

Here we are confirm that the article entitled :

" Prenatal and Neonatal Factors Related With Autism Spectrum Disorder : a case control study in Banyumas, Central Java, Indonesa"

by the authors : Desiyani Nani, Elisabeth Siti Herini, Ahmad Hamim Sadewa, Sri Hartini, Lita Heni Kusumawardani, Dyla Annisa Putri has been submitted to our journal partner **" Annals of Tropical Medicine and Public Health.** The next decision awaits the results of the journal

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Prenatal and Neonatal Factors Related With Autism Spectrum Disorder: a case-control study in Banyumas, Central Java, Indonesia

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ABSTRACT

Introduction: Autism spectrum disorder (ASD) is a complicated mental disorder and the etiology is still unknown. Several studies showed the possibilities of prenatal and neonatal factors becoming risks for ASD. This study aimed to identify the possibilities of risk factors in prenatal and neonatal condition that could be related with ASD.

Methods: This research was a case control study in the Banyumas District Area, Province of Central Java, Indonesia. It included 52 children with ASD as the case group and 201 normal children as the control group. Data were collected with demographic tools with questions about prenatal and neonatal factors. Analyzed data used Chi-Square, Fisher Exact tests, and Regression Logistic analyses.

Results: This study found that father with active smoking, high-income in socio-economic status, mother with passive smoking during pregnancy, multipara mother, and history of neonatus CPR significantly increased the risk to have children with ASD (p=0.000, OR: 0.256; p=0.000, OR: 3,23; p=0.032; OR:0.490; p=0.019, OR: 2.15; p=0.000; OR:11.760). Other factors such as history of antenatal care, history of gestation complication and newborn weight were not significantly correlated with ASD (p>0.05).

Conclusions: Father with active smoking, high income in socio-economic status, mother with passive smoking during pregnancy, multipara mother and history of neonatus CPR were risk factors for ASD.

Recommendations: Additional studies are needed to identify the possibilities of other prenatal and postnatal factors in ASD.

Keywords: Prenatal, Neonatal, and autism spectrum disorder, ASD risk factors

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INTRODUCTION

Autism belongs to a spectrum known as autism spectrum disorders (ASDs), which includes Asperger's syndrome, childhood disintegrative disorder, and pervasive developmental disorder not otherwise specified[1]. ASDs present with a range of severity and impairments in social, communication skill, behavior and interest[2]. Additionally, most individuals with autism also present with intellectual and cognitive impairments[3,4]. There are

more than 7.6 million people with ASD accounting for 0.3% of the global burden of disease[5]. On the other hand, the number of ASD in developing countries like Indonesia is not exactly unknown and it is reflected by fewer studies about ASD[6]. The etiology of ASD is diverse, commonly still unknown, and seems to be the result of genetic and environmental interaction[3,2]. Some previous studies showed that genetic factors account for only 35-----40% of the contributing elements. The remaining 60---65% are likely due to other factors, such as prenatal, perinatal, and neonatal environmental factors. Unfavorable enviromental factors during pregnancy and neonatal period are likely causes for brain damage, which is one of the prescribed risk factors of ASD[2]. Several studies show prenatal and neonatal factors related with ASD. Those factors are maternal age, paternal smoking, maternal smoking, complication during gestation, child's birth weight, and hypoxia[7,8,9]. Considering the potential risk about perinatal factors, this research conducted a case control study to investigate the association between prenatal and neonatal factors with ASD. This study investigated some prenatal factors such as maternal age, paternal smoking, history of passive smoke during pregnancy, socio-economic status, history of antenatal care, history of complications during gestation, and parity status. History of newborn weight and history of neonatus cardiopulmonary resuscitation (CPR) were also investigated in this research.

METHODS

Study setting and design

This case control study was conducted at a community in Banyumas District, Province Central Java, Indonesia, over a period of four months from October 2017 to January 2018.

Study Population

The sample included 253 children in Banyumas District, Province of Central Java, Indonesia. Subjects were divided into case group and control group. The case group was composed of 52 autistic children previously identified by a psychologist in the Banyumas Autism Care Project (BACP) event. The control group was composed of 201 normal children. Inclusion criteria were children with age between 3-18 years old and willing to be respondents, while the exclusion criteria were children with known neurogenetic conditions (e.g., Down Syndrome, Mental Retardation, Neurofibromatosis).

Sampling design

Sample size

Using sex differences between ASD within boys and girls as a risk factor and to detect an odds ratio (OR) of at least 2 with a power of 80% using a 5% level of significance, the sample size was calculated to be 52 cases and 201 controls.

Sample type and selection

Consecutive cases were selected over 4 months, with every weekend allocated to visiting the school of children with special needs. We invited parents who have children with ASD who independently accepted and confirmed the informed consent to permit their children to become participants for this study. The location for sampling

was in the Research Center of the Faculty of Medicine, Universitas Jenderal Soedirman, Banyumas District, Central Java Province, Indonesia.

Controls were selected from normal children who came with their parents and were also visited by our team at local schools, till the required sample size was reached.

Measurements

This study used a demographic tool with questions about perinatal history. It consisted of 9 questions about: 1) maternal age, 2) paternal smoking, 3) history of smoking during pregnancy, 4) socio-economic status, 5) history of antenatal care, 6) history of complication during pregnancy, 7) parity status, 8) history of newborn weight, and 9) history of neonatus cardio pulmonary resucitation (CPR).

Data Collection

Over four month data were collected from parents of children ages 2-18 years old with significantly diagnosed using DSM 4 and DSM 5 by professional practitioners then grouped into the case group (children with ASD) and the control group (children in normal state) using the questionnaire detailing demographic and risk factors for ASD.

Statistical Analysis

Analyses were first run to describe the data and then to describe the trends resulting from each item. Bivariate analysis used Chi-square tests to explore correlations between variables. This study also used logistic regression as multivariate analyses to identify the most affecting factors in ASD.

Ethical considerations

Consent was obtained from all parents (mothers/fathers) after an explanation of the study objectives was provided and anonymity of the results was assured. Approval for the study was obtained from Medical and Health Research Ethics Committee of the Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia.

RESULTS

Table 1. Bivariate Analysis Results

		Case Group (n= 52)		Control Group (n = 201)		*p*	OR	CI95%	
		n	%	n	%			Min	Max
Maternal Age	<20 years	3	5,8	24	11,9	0,332	1,87	0,527	6,639
	21-30 years	29	55,8	124	61,7		ref		
	31-40 years	19	36,5	53	26,4	0,115	2,87	0,774	10,63
	>40 years	1	1,9	0	0				

Paternal Smoking	Active smoking	14	26,9	114	56,7	0,000	3,89	1,957	7,769
	Passive smoking	4	7,7	16	8	0,257	2,036	0,569	6,952
	Not smoking	34	65,4	71	35,3	ref			
History of smoking during pregnancy	Passive smoking	15	28,8	91	45,3	0,032	0,49	0,253	0,949
	Not smoking	37	71,2	110	54,7	ref			
Socio-Economic Status	>Rp 1.549.000,00	37	71,2	87	56,7	0,000	3,23	1,67	6,26
	<Rp 1.549.000,00	15	28,8	114	43,3	ref			
History of Antenatal Care	Not annual	1	1,9	8	4	0,475	2,11	0,26	17,29
	Annual	51	98,1	193	96	ref			
History of Complication During Pregnancy	Present	28	46,2	84	41,8	0,119	1,63	0,88	3,00
	Not Present	24	53,8	117	58,2	ref			
Parity Status	Primipara	18	34,6	102	50,7	ref			
	Multipara	33	63,5	87	43,3	0,019	2,15	1,13	4,08
	Grande-Multipara	1	1,9	12	6	0,484	0,47	0,06	3,86
History of Newborn Weight	<2500	0	0	5	2,5	0,999	0,00	-	-
	2500-4000	49	94,2	191	95	ref			
	>4000 gr	3	5,8	5	2,5	0,256	0,43	0,1	1.85
History of Neonatus Cardio Pulmonary Resuscitation (CPR)	Yes	12	23,1	5	2,5	0,000	11,76	3,93	35,23
	No	40	76,9	196	97,5	ref			

This study included 253 parents, from whom 52 were cases (parents with ASD children) and 201 were control (parents with normal children). Table 1 shows that mothers with age lower than 20 years during pregnancy had almost two times greater (OR: 1.87) risk to have children with ASD. Mothers with age between 31-40 years also had increased risk of ASD by almost three times (OR: 2.87). However, this maternal age category was not significantly correlated with ASD. Considering the smoking status, when the father was an active smoker, the risk of ASD increased almost four times (OR: 3.89) and it was significantly correlated with ASD (p=0.000). The factor of father as passive smoker also had increased risk for ASD by two times, but it was not significantly correlated with ASD. Mothers as passive smoker were also correlated with ASD (p=0.032) but with a low OR value (OR:0,49).

Considering the socio-economic status, 71.2% of the parents in the case group had income above the regional minimum salary (> Rp 1,589,000.00). Comparing with the control group, only 56.7% of theparents in the

control group had income above the regional minimum salary. Parents with income above minimum region salary (>Rp 1,589,000.00) had a risk three times greater to have children with ASD (OR: 3.23, CI 95%: 1.67-6.26) and this finding was significantly correlated with ASD (p=0.000).

Mothers who didn't do routine antenatal care during pregnancy also had increased risk of ASD by more than two times (OR: 2.11, CI 95%: 0.26-17.29), but it was not statistically significantly correlated with ASD (p=0.475). Mothers with complications during pregnancy such as anemia, hypertension, hyperemesis, etc. did not have significant risk to have children with ASD (p=0.119; OR: 1.63; CI 95%: 0.88-3.00). Multipara mothers with two children or more were also significantly correlated with ASD (p=0.019) and had two times greater risk to have children with ASD (OR: 2.15, CI 95%: 1.13-4.08). Comparing with multipara mothers, grande multipara mothers with more than four children were not correlated with ASD (p=0.484) and also had a lower OR value (OR: 0.47, CI 95%: 0.06-3.86).

Table 1 also shows the correlations between neonatal factors with ASD. Children with history of low newborn weight (<2.500 gr) or macrosomia (>4.000 gr) were not significantly correlated with ASD and did not have a risk of ASD. The factor of history of neonatus CPR showed a higher percentage in the case group (23.1%) compared to the control group (2.5%). It also had a strong correlation with ASD (p=0.000) with 12 times increased risk of ASD.

Table 2. Final Result of Multivariate Analysis with Logistic Regression Model

Variable	S.E.	Sig.	OR	CI 95%	
				Min	Max
History of neonatus CPR	0.628	0.000*	10.78	3.151	36.883
Paternal smoking	0.387	0.000*	3.872	1.814	8.268
Socio-economic status	0.370	0.008*	2.665	1.290	5.504
Parity status	1.197	0.036*	12.249	1.174	127.82

*$p<0.05$

Table 2 shows the final result of logistic regression model of the significant risk factors correlated with ASD. After adjusting for prenatal and neonatal factors, it extracted four factors as the most significant predictors of ASD: history of neonatus CPR, paternal smoking, socio-economic status, and parity status. A higher risk of ASD was significantly correlated with a positive history of neonatus CPR (OR: 10.78; CI 95%: 3.151-36.883), father as active smoker (OR: 3.872; CI 95%: 1.814-8.268), higher socio economic status (OR: 2.665; CI 95%: 1.29-5.504), and multipara mother (OR: 12.249; CI 95%: 1.174-127.82).

DISCUSSIONS

There was a potential risk for mothers with age lower than 20 years during pregnancy and age between 31-40 years to have children with ASD. Maternal ages above 30 years were associated with a greater risk of ASD with intellectual disabilities with pooled OR at 2.04 (CI 95%: 1.82–2.30)[10].Mothers with age above 30 years also had a risk almost two times compared with mother with age below 30 years [11] (OR: 1,80, CI95%: 1,27-2,54). However, maternal age was not significantly correlated with ASD.[12,13]Father as active smokers increased the risk of ASD almost four times (OR:3.89) and was significantly correlated with ASD (p=0.000). The factor of father as passive smoker also increased the risk of ASD by two times, but it was not significantly correlated with ASD. This finding is consistent with previous research [14] that found a strong relationship between fathers as active smokers with ASD (p=0.007) and the risk increased almost three times (OR: 2.6; CI 95%: 1.3-5.2). Despite this result, only a few studies are available that investigate about paternal smoking with ASD and made a lack of mechanism of this variable.Mother as passive smoker was also correlated with ASD (p=0.032) but it had a low OR value (OR:0.49, CI 95%: 0.253-0.949). This study also found that there were no active smoking mothers during pregnancy. Several previous studies show a potential risk of maternal smoking with ASD. [15]Exposure to cigarette smoke during pregnancy is associated with increased risk of ASD with p<0.000. [16] Passive smoking during pregnancy would increase the risk of ASD three times and it had strong relationship with p=0.005 and OR: 2.57, CI 95%: 2.57-1.23-5.36. Compared with active smoking, passive smoking is considered more dangerous and potentially can cause ASD by its exposure.Parents with income above the regional minimum salary (>Rp 1,589,000.00) had a risk three times greater to have children with ASD (OR: 3.23, CI 95%: 1.67-6.26) and it was significantly correlated with ASD (p=0.000)[17]. Prevalence of ASD with mild impairment increases with income, with prevalence of mild autism significantly higher in the top two income categories than in the lower two (from lowest to highest income, cases per 1000 (95% CI): 1.8 (1.2–2.9); 2.2 (1.8–2.8); 4.6 (3.5–6.2); 5.9 (4.2–8.4), respectively)[18]. Most of children with ASD came from a high income family. This finding is surprising and still needs more investigation to understand the underlying mechanism between socio-economic status and ASD. Mothers without routine antenatal care visits during pregnancy also had increased risk of ASD by two times (OR: 2.11, CI95%: 0,26-17,29), but it was not statistically significantly correlated with ASD (p=0.475). The next finding also reported mothers with complications during pregnancy such as anemia, hypertension, hyperemesis, etc. did not have significant risk to have children with ASD (p=0.119; OR: 1.63; CI 95%: 0.88-3.00)[18]. Other studies have shown that there were no correlations between complications such as gestational infection or diabetes mellitus with ASD[16]. There were also no associations between other pregnancy complications like preeclampsia, fever, or diabetes with ASD. These complications during pregnancy appear to not affect ASD but the underlying mechanism is still uncertain.

In this present study, multiparous mothers with two children or more were significantly correlated with ASD (p=0.019) and had two times more risk to have children with ASD (OR: 2.15, CI 95%: 1.13-4.08). [19]Another study found the same result that pregnancy frequencies between two and three were associated with ASD and increased risk of ASD by two times. [8] Most mothers with ASD children had a parity status of more than one and it was correlated with ASD. However, some studies show abstinence significant value of these factors with ASD[20,16]. Investigation of neonatal aspects and their correlation with ASD in this study identified newborn weight and neonatus CPR as significant risk factors. Children with history of low birth weight (<2.500 gr) or macrosomia (>4.000 gr) did not have significant correlation with ASD and were not at risk for ASD. The same

result was shown from several studies that reported there was no association between birth weight with ASD[16,11,21]. However, different results from a meta-analysis study reported low birth weight (<2000 gr) was associated with ASD and had two times increased risk for ASD (OR: 2,20, CI 95%) [22]. Low birth weight was considered a predictor for poor brain development and it was associated with risk factors of ASD. Oppositely, this present study found no significant correlation between birth weight and ASD. These findings need further study to identify and explain a clear mechanism between birth weight and ASD. This present study identified another neonatal factor, neonatus CPR which had a strong association with ASD (p=0.000) and almost 12 times increased risk for ASD (OR: 11.76, CI 95%: 3.95-35.23). Logistic regression analyses also showed this factor was the greatest risk of ASD (p=0.000,OR: 11.78). Several previous studies showed neonatus resuscitation was the greatest risk for ASD. [11] Children with history of neonatus CPR due to delayed crying or birth asphyxia had 11 times greater risk for ASD. [23,13] Infant hypoxia can trigger neonatus CPR and is significantly correlated with ASD. The fact that neonatus CPR became the greatest risk of ASD may be caused by the late oxygen and blood delivery to the infant's brain. This mechanism is a well-known reason for poor brain development and triggers the risk of ASD[24].

Conclusions

This present research is consistent with several previous studies about prenatal and neonatal risk factors in ASD. The findings indicate neonatus CPR, paternal smoking, socio-economic status, and parity status are strongly associated with ASD. Children with prenatal and neonatal risk factors should be early examined by thorough medical tests to increase the effectivity of therapies. This study also being the first representation from a rural society, especially in Indonesia and becomes noteworthy.

Study Limitations

This present study had several limitations. Complications during pregnancy as a prenatal factor must be split into several variables to be described clearly. Parental nutritional status during pregnancy also could be investigated as a prenatal risk factor for ASD. Another cohort study about risk factors for ASD in Indonesia is also badly needed.

Acknowledgments

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