Original Article

Association between Autism Spectrum Disorder and Breastfeeding Initiation and Duration: A Case-Control Study in Sulaymaniyah, Iraq

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Submission: January 21, 2026 Accepted: February 26, 2026 Published: March 31, 2026

Abstract

Background: Autism Spectrum Disorder (ASD) is a multifactorial neurodevelopmental condition influenced by genetic, perinatal, and environmental factors. Although breastfeeding has been proposed as a potential protective factor for neurodevelopment, evidence remains inconsistent across populations. **Objectives:** This study aimed to assess the association between breastfeeding practices and ASD among children in the Sulaymaniyah Governorate, Kurdistan Region of Iraq. **Materials and Methods:** A case-control study was conducted from August 2024 to February 2025 involving 222 children aged 2–15 years (77 ASD cases and 145 controls). ASD diagnoses were confirmed by a specialized multidisciplinary committee using DSM-5 criteria. Data were collected through a validated semi-structured questionnaire covering sociodemographic, prenatal, perinatal, and breastfeeding variables. Statistical analyses were performed using SPSS v23.0; Chi-square, t-tests, and Mann–Whitney U tests were applied where appropriate. **Results:** No statistically significant association was found between ASD and breastfeeding initiation, exclusivity, or duration. Ever-breastfed proportions were similar between cases and controls (93.5% vs 88.3%, p = 0.214). **Conclusion:** Breastfeeding practices did not show a significant relationship with ASD in this regional cohort. The findings highlight that parental age, family history, and educational and occupational factors may exert a stronger influence on ASD risk than infant feeding patterns. Further large-scale prospective studies are warranted to clarify causal mechanisms.

Keyword: Autism Spectrum Disorder; Breastfeeding; Breastfeeding Duration; Neurodevelopment; Maternal Age.

Introduction

Autism is a condition that affects a child's ability to communicate and interact with other people, as well as to display interest in repetitive or stereotyped behaviors. Individuals with autism have restricted and repetitive behavior patterns, that may hinder their capacity to participate in common social interactions [1]. In 2020, the Centers for Disease Control and Prevention (CDC) estimated that about one in 36 children aged 8 years had an ASD, approximately 22,2% increase from 2018 [2]. The cause of ASD is not known exactly, but several theories have been put forward. It is known that ASD has a genetic component, environmental influences, or both, which interfere with neurological development [3]. However, there is no environmental factor that has been shown to directly cause autism; rather, it is thought that a number of factors may contribute to the disease's development [4]. Prevention of ASD is still a difficult task due to the complex origins of the disorder. Breastfee-

ding has been suggested as a possible protective environmental factor against ASD. Breastfeeding is socially interactive and a dynamic process occurring simultaneously between the mother and infant as both become mutually engaged during the process itself. Studies showed that breastfed infants have a reduced risk of various childhood illnesses and better overall health outcomes [5,6]. There have been proposed various mechanisms that may contribute to breastfeeding and ASD prevention, such as nutritional benefits [7], stimulation of oxytocin release [8], and the presence of certain neurotrophic substances in breastmilk which might support healthy neurological growth [9]. There have been mixed findings in past researches on the relationship between ASD and breastfeeding. Most studies indicate that breastfeeding may have protective effect against ASD and is related to less autistic traits [7,10–13]. Some researchers also indicates that the risk of ASD may be lowered by extended breastfeeding [7,11,14,15]. Nevertheless, there are studies that have not reported a significant association between breastfeeding and the condition [16-18]. A meta-analysis of 38 studies with 149,607 individuals found that non-exclusive breastfeeding was associated with significantly increased risk of ASD (OR, 2.51; 95% Cl, 1.85-3.40, $p < 0.001$), formula feeding or mixed feeding was associated with increased risk of ASD compared with exclusive breastfeeding [19]. The results from these findings suggest that exclusive breastfeeding may have a protective role in neurodevelopment. Ghozy et al, have found that ever breastfeeding was associated with 58% reduced risk of ASD (OR = 0.42, 95% CI: 0.30-0.57) and exclusive breastfeeding, 76% reduced risk of ASD (OR = 0.24, 95% CI: 0.18-0.32) [14]. However, the findings suggest that there is a dose response effect and that prolonged breastfeeding beyond six months may contribute to a greater reduction in ASD risk. A case control study in Brazil found that children who were not breast fed had a significantly higher risk of ASD (adjusted odds ratio 2.1 (95% Cl 1.1–4.1, p= 0.025)) [20]. However, breastfeeding duration alone did not significantly relate to ASD. Nonetheless, a large case control study from the Study to Explore Early Development (SEED) did not find an association between ASD risk and breastfeeding initiation, but an association between longer breastfeeding duration (≥12 months) and a lower risk of ASD (aOR: 0.61 95% Cl: 0.45-0.84) [15].

The current study is the first attempt to investigate the association between breastfeeding and ASD development in Iraq Using Case-Control Design.

Materials and Methods

The sample size was calculated based on the prevalence of exclusive breastfeeding among the general population, estimated at 24,9% (P_1 = 0.249) and among children with ASD at 10.1% (P_2 = 0.101), and the difference between the two is 14.8% (P_1 - P_2 = 0.148). In this study, the statistical power was set at 0.80 and the significance level was at 0.05 ($Z\alpha$ = 1.96) to ensure statistical reliability. The sample size was calculated for case control studies using the appropriate formula and a case to control ratio of 1:2 was applied. It was determined that 73 cases were required, and 77 were rounded up for the study's power. According to the 1:2 ratios, the sample size of the control group was set to 145, giving the total sample size of 222 participants. The case group was selected from people who went to the Autism Detection Committee at the Directorate of Health (DOH) in Sulaymaniyah. The committee meets weekly and assembles to assess and confirm or rule out ASD children. All cases that were investigated were diagnosed

according to the *Diagnostic and Statistical Manual of Mental Disorders* – 5 (DSM-5) criteria [1], by a specialized committee of psychiatrists and pediatricians. For the purpose of the study, only children with confirmed diagnosis of ASD by the committee were recruited. The participants, regardless of gender, were children 2 to 15 years of age. To minimize recall bias concerning history of breastfeeding, participants older than 15 years were excluded. Moreover, to help reduce the potential for confounders, children with deafness, neuropsychiatric disorders or systemic and genetic conditions, including Fragile X syndrome, were also excluded. Neurotypical children attending the Pediatrics Teaching Hospital for routine health checkups or minor, non-chronic conditions constituted the control group. Controls were recruited during the same study period and from the same healthcare facilities as the ASD cases to ensure that they represented the source population from which the cases arose and were subject to similar healthcare access and referral patterns. Children within the same age range as the case group (2–15 years) were eligible for inclusion. The control group comprised children presenting with minor acute conditions, such as common colds, or those undergoing routine physical examinations. Children with a diagnosis or clinical suspicion of Autism Spectrum Disorder, other neurodevelopmental disorders, genetic syndromes (including Fragile X syndrome), or significant neurological impairment were excluded. The absence of ASD and other neurodevelopmental conditions among controls was confirmed through medical record review and parental report at the time of enrollment. Controls were not individually matched to cases; however, potential confounding by age and sex was addressed through adjustment in multivariable logistic regression analyses. The case and control groups were interviewed between August 2024 and February 2025. A semi-structured questionnaire was used as the primary data collection tool. It was developed after a literature review [21,22] and it was reviewed by a multidisciplinary team for clarity and relevance. Seven sections were covered in the questionnaire, including characterization of the subject, demographics, pregnancy and birth details, breastfeeding history, maternal factors during pregnancy, perinatal factors (e.g. birth complications, NICU admission), and socioeconomic information. The exposure variables analyzed in this study included Breastfeeding-related Variables included whether or not the child was ever breastfed, duration of exclusive breastfeeding, duration of any breastfeeding (exclusive or not), early initiation of breastfeeding, age of introduction of formula feeding and complementary feeding and breastfeeding patterns (predominantly during the day or night). In order to control for possible confounding factors, additional variables were examined, including maternal and paternal age at childbirth, birth order, family history of ASD, gestational age at birth, birth weight, presence of twin pregnancy, type of delivery, any maternal disease or complications during pregnancy, maternal obesity before pregnancy, prenatal vitamin and vitamin D supplementation, frequent use of paracetamol during pregnancy, NICU admission, cyanosis at birth, presence of jaundice after birth, presence of meconium, parental education level at the time of childbirth, type of residency, employment status at the time of childbirth, and family socioeconomic status.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 23.0 (IBM Corp., Chicago, USA). Descriptive statistics were expressed as means ± standard

deviations (SD) for continuous variables and as frequencies and percentages for categorical variables. The normality of data distribution was assessed using the Kolmogorov–Smirnov test. For comparisons between the ASD and control groups, the independent sample t-tests was applied for normally distributed continuous variables, while the Mann–Whitney U test was used for non-normally distributed data. Chi-square tests or Fisher's exact tests (when expected frequencies were <5) were employed to compare categorical variables. Variables with a p-value ≤0.20 in the bivariate analysis were entered into a multivariate logistic regression model using a backward stepwise approach to identify independent predictors of ASD. To assess potential effect modification, interaction terms between breastfeeding-related variables and child sex were included in the multivariate logistic regression models. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. Statistical significance was defined as a two-tailed p-value <0.05.

Ethical Approval

This study received approval from the Research Ethics Committee of the University of Sulaimani, College of Medicine (Protocol No. 119), on 3 January 2024. Written informed consent was obtained from the guardians of all participating children and adolescents.

Results:

A total of 222 participants were included in the study with no missing data. Of these, 77 (34.7%) were classified as ASD cases and 145 (65.3%) as controls. Baseline characteristics are presented in Table 1. There were no statistically significant differences were detected between the groups in key child characteristics, including gender distribution (p = 0.810), child's age (p = 0.152), and gestational age at birth (p = 0.813). A positive family history of ASD or related neurodevelopmental conditions was more frequent among cases than controls (33.8% vs. 20.7%, p = 0.033). However, the prevalence of ASD among siblings did not differ significantly between the two groups (p = 0.502). Both maternal and paternal ages were significantly higher in the ASD group (p < 0.001 for mothers and p = 0.004 for fathers). Paternal education level also differed significantly between groups (p = 0.003), with lower education levels more common among fathers of children with ASD. Maternal education did not show a statistically significant difference (p = 0.247). Most pregnancy variables did not significantly differ between ASD and control groups. Vitamin D supplementation during pregnancy showed borderline significance (p = 0.085), as did postpartum depression or anxiety (p = 0.058). No statistically significant associations were observed for prenatal vitamin use (p = 0.092), iron supplementation (p = 0.198), paracetamol use (p = 0.115), or other pregnancy complications.

Table 1: Baseline Child, Parental, and Pregnancy Characteristics of ASD Cases and Controls

Variables		Cases (n=77)	Controls (n=145)	*P-Value*
		n (%) or Mean ± SD / Median (IQR)	n (%) or Mean ± SD / Median (IQR)	
Child characteristics	Gender			
	Male	59 (76.6%)	109 (75.2%)	0.810
	Female	18 (23.4%)	36 (24.8%)	

	Child's Age (months)			
	Mean ± SD / Median (IQR)	86.77 ± 32.05 / 80.0 [63.0–105.5]	79.05 ± 40.88 / 67.0 [45.0–111.5]	0.152
	Gestational Age at Birth (weeks)			
	Mean ± SD / Median (IQR)	38.43 ± 2.19 / 39.0 [38.0–40.0]	38.50 ± 1.96 / 39.0 [38.0–40.0]	0.813
Family psychiatric/genetic history	**Family History of ASD or Similar Conditions**			
	Yes	26 (33.8%)	30 (20.7%)	0.033
	No	51 (66.2%)	115 (79.3%)	
	Siblings' History of ASD or Similar Conditions			
	Yes	4 (5.2%)	5 (3.4%)	0.502
	No	73 (94.8%)	140 (96.6%)	
Parental socio-demographic factors	**Maternal Education at Birth**			
	No Formal Education	5 (6.5%)	11 (7.6%)	0.247
	Primary Education	22 (28.6%)	47 (32.4%)	
	Secondary Education	17 (22.1%)	41 (28.3%)	
	Higher Education	33 (42.9%)	43 (29.7%)	
	Paternal Education at Birth			
	No Formal Education	2 (2.6%)	6 (4.1%)	0.003
	Primary Education	26 (33.8%)	57 (39.3%)	
	Secondary Education	15 (19.5%)	31 (21.4%)	
	Maternal Age (years)			
	Mean ± SD / Median (IQR)	31.81 ± 6.08 / 32.0 [27.5 – 37.0]	28.63 ± 6.19 / 29.00 [24.00 - 34.00]	<0.001
	Paternal Age (years)			
	Mean ± SD / Median (IQR)	36.6 ± 7.69 / 36.0 [32.0 - 40.5]	33.40 ± 6.99 / 33.0 [29.0 38.0]	0.004
Pregnancy characteristics	**Use of Vitamin D Supplement During Pregnancy**			
	Yes	39 (50.6%)	75 (51.7%)	0.085
	No	25 (32.5%)	59 (40.7%)	
	Did Not Remember	13 (16.9%)	11 (7.6%)	

	Prenatal Vitamin Supplementation			
	Yes	68 (88.3%)	112 (77.2%)	0.092
	No	8 (10.4%)	32 (22.1%)	
	Did Not Remember	1 (1.3%)	1 (0.7%)	
	Prenatal Iron Supplementation			
	Yes	27 (35.1%)	69 (47.6%)	0.198
	No	45 (58.4%)	69 (47.6%)	
	Did Not Remember	6 (6.5%)	7 (4.8%)	
	Postpartum Depression or Anxiety			
	Yes	19 (24.7%)	54 (37.2%)	0.058
	No	58 (75.3%)	91 (62.8%)	

Note: Categorical variables are expressed as n (%). Continuous variables are presented as mean ± SD and median (IQR). Group differences were assessed using Chi-square or Fisher's exact test for categorical variables and independent t-tests or Mann–Whitney U tests for continuous variables according to distribution. Statistical significance was set at $p < 0.05$. Significant values are shown in bold.

Table 2 summarizes the breastfeeding patterns and practices among children with ASD (cases) and the control group. No statistically significant differences were detected between the two groups with respect to whether the child was ever breastfed, timing of breastfeeding initiation, breastfeeding patterns, or the primary reasons for stopping breastfeeding (all $p > 0.05$). Similarly, the age at introduction of complementary feeding, age at formula milk introduction, and weaning age did not differ significantly between the groups. Moreover, measures of breastfeeding duration including exclusive, partial, and any breastfeeding showed no statistically significant differences between the two groups.

Table 2: Breastfeeding Patterns and Practices among Case and Control Groups (N=222).

Variables		**Cases (n=77)**	**Controls (n=145)**	***P-Value***
		n (%) or Mean ± SD/ Median (IQR)	**n (%) or Mean ± SD/ Median (IQR)**	
Categorical variable	**Was the Child Ever Breastfed**			
	Yes	72 (93.5%)	128 (88.3%)	0.214
	No	5 (6.5%)	17 (11.7%)	
	When Was Breastfeeding Initiated			
	Within the First Hour	21 (27.3%)	47 (32.4%)	0.077
	Within 1-24 Hours After Birth	30 (39.0%)	59 (40.7%)	
	More Than 24 Hours After Birth	21 (27.3%)	22 (15.2%)	
	Did Not Breastfeed	4 (5.2%)	17 (11.7%)	

	Missing Data	1 (1.3%)	0 (0.0%)	
	Breastfeeding Pattern			
	Mainly During the Day	19 (24.7%)	23 (15.9%)	0.115
	Mainly During the Night	8 (10.4%)	18 (12.4%)	
	Both Day and Night Equally	46 (59.7%)	77 (53.1%)	
	Did Not Breastfeed	4 (5.2%)	18 (12.4%)	
	Primary Reason for Stopping Breastfeeding			
	Insufficient Milk Supply	17 (22.4%)	30 (20.7%)	0.075
	Return to Work	1 (1.3%)	1 (0.7%)	
	Health Issues (Mother/Child)	0 (0.0%)	1 (0.7%)	
	Child Weaned Naturally	3 (3.9%)	0 (0.0%)	
	Baby's Preference	1 (1.3%)	4 (2.8%)	
	Nutritional Needs	3 (3.9%)	21 (14.5%)	
	Cultural or Social Pressures	6 (7.9%)	16 (11.0%)	
	Other	15 (19.7%)	15 (10.3%)	
	Did Not Remember	15 (19.7%)	35 (24.1%)	
	Duration of Exclusive Breastfeeding			
	Never breastfed	4 (5.2%)	17 (11.7%)	0.365
	Breastfed	27 (35.1%)	61 (42.1%)	
	exclusive Exclusive breastfed (various	46 (59.7%)	67(46.2)	
Continuous Variable	**Age at Introduction of Complementary Feeding (weeks)**			
	Mean ± SD	27.92 ± 9.61	24.32 ± 5.24	0.321
	Median (IQR)	26.0 [26.0 – 26.0]	26.0 [22.0 – 26.0]	
	Age at Introduction of Formula Milk (weeks)			
	Mean ± SD	39.40 ± 37.43	36.71 ± 33.41	0.665
	Median (IQR)	26.0 [11.0 – 76.0]	26.0 [13.0 – 55.5]	
	Weaning Age from Breastfeeding (weeks)			
	Mean ± SD	74.60 ± 43.08	81.21 ± 40.53	0.448
	Median (IQR)	82.0 [35.0 – 104.0]	104.0 [39.0 – 108.0]	

	Duration of Exclusive Breastfeeding (weeks)			
	Mean ± SD	19.28 ± 12.82	17.79 ± 7.95	0.669
	Median (IQR)	17.0 [11.0 – 26.0]	19.5 [13.0 – 26.0]	
	Duration of Partial Breastfeeding (weeks)			
	Mean ± SD	55.96 ± 39.16	63.46 ± 39.89	0.520
	Median (IQR)	52.0 [19.5 – 85.5]	80.0 [22.3 – 95.0]	
	Duration of Any Breastfeeding (weeks)			
	Mean ± SD	74.60 ± 43.08	81.21 ± 40.53	0.487
	Median (IQR)	82.0 [35.0 – 104.0]	104.0 [39.0 – 108.0]	

Note: P-values were calculated using Chi-square or Fisher's exact test for categorical variables and t tests or Mann-Whitney U tests for continuous variables. Statistical significance was set at P < 0.05, with significant values highlighted in bold.

In Table 3, none of the variables included in the multivariate logistic regression model were identified as statistically significant predictors of ASD (p > 0.05). Maternal age ≥35 years demonstrated a non-significant trend toward increased odds of ASD (OR = 4.363; 95% CI: 0.822–23.150; p = 0.084). Ever breastfeeding was not associated with ASD (OR = 0.92; 95% CI: 0.70–1.21; p = 0.56), and no statistically significant interaction was detected between breastfeeding and child sex (p = 0.48). Age at formula milk introduction (OR = 0.978; 95% CI: 0.952–1.004; p = 0.102) also showed near-statistically significant association. All other predictors, including family history of ASD, paternal age, vitamin D use during pregnancy, maternal drug intake, and duration of any breastfeeding, were not statistically significant in this sample.

Table 3: Predictors of Autism Spectrum Disorder (ASD) in Multivariate Logistic Regression Analysis (N=222)

Predictor	Odds Ratio (95% Confidence Interval)	P-value
Was child ever breastfed (yes vs no)	0.92 (0.70 – 1.21)	0.560
Child sex (Male vs Female)	0.78 (0.60 – 1.02)	0.070
Ever breastfeeding * child sex	1.12 (0.81 – 1.56)	0.480
Age at formula milk introduction (continuous)	0.98 (0.95 – 1.00)	0.102
Use of vitamin D during pregnancy	2.19 (0.68 – 7.06)	0.188
Maternal other drug intake	0.65 (0.17 – 2.40)	0.512
maternal age at pregnancy > 35 years (yes vs no)	4.36 (0.82 – 23.15)	0.084
Paternal age at pregnancy > 35 years (yes vs no)	0.95 (0.23 – 3.88)	0.937

Positive family history of ASD (yes vs no)	0.96 (0.19 – 4.90)	0.961

Note: The multivariable logistic regression model included ever breastfeeding (yes vs no), child sex, an interaction term between breastfeeding and child sex, parental ages, family history of ASD, prenatal vitamin D use, maternal drug intake, and age at formula milk introduction. OR = odds ratio; CI = confidence interval. None of the predictors in this model were statistically significant ($p > 0.05$).

Discussion

This case-control study investigated the relationship between breastfeeding practices (initiation and duration, including exclusive, partial, mixed breastfeeding) and autism spectrum disorder (ASD) among children residing in Sulaymaniyah, Kurdistan Region of Iraq. No statistically significant associations between breastfeeding practices and ASD were detected in this study. A study conducted in Egypt concluded that there was no significant difference in breastfeeding practices between children with ASD and their typically developing siblings, despite early initiation of breastfeeding was associated with less severe symptoms of ASD and better intellectual outcomes [23]. A U.S national survey of children's health found no significant differences in duration of breastfeeding between neurotypical and ASD children, but Initiation was not considered a variable [16]. Nevertheless, a large community case-control study did not find any significant associations regrding initiation after adjustment for confounding variables, whereas duration emerged as significant factor destingusing Breastfeeding and ASD in Children with ASD and typically developing children [15]. Some of These findings are in line with the current study, in which no statistically significant effect of breastfeeding practices on ASD development was detected. In contrast, other studies suggested that breastfeeding; particularly exclusive breastfeeding may have protective effects. A systematic review and meta-analysis on breastfeeding and neurodevelopment concluded exclusive breastfeeding during the first six months was associated with a lower risk of ASD (OR = 0.55, 95% CI: 0.40–0.76, $P < 0.001$) [11]. Regional studies have reported a lower prevalence of exclusive breastfeeding, among children with ASD, including reports from Lebanon and Saudi Arabia; However these studies emphasized the need for further research to clarify causal relationships [10,24]. In addition to breastfeeding practices, a comprehensive analysis of various demographic, perinatal, and maternal variables was conducted to identify potential confounding factors. Notably, none of these variables exhibited statistically significant associations with ASD in either univariate or multivariable analyses (Tables 1–3). While these factors were not the primary exposures of interest, their inclusion facilitated the adjustment of potential confounding and enhanced the validity of the estimated association between breastfeeding practices and ASD.

Limitations

This study has several limitations that should be acknowledged. First, the retrospective case–control design relied on maternal recall of infant feeding practices, which may have introduced recall bias, particularly regarding breastfeeding duration and exclusivity. Second, the hospital-based selection of controls may limit the generalizability of the findings to the broader population. In addition, some potential confounding factors, including maternal psychiatric history and parental consanguinity, were not assessed and may have contributed to residual confounding. Although interaction analyses were performed to explore effect modification by child sex, the relatively modest

sample size may have limited the ability to detect subtle interaction effects. Finally, despite adjustment for multiple confounders using multivariable logistic regression, no statistically significant associations were detected. This may reflect limited statistical power, possibly due to the modest sample size, rather than a true absence of association.

Conclusion

In this study, no statistically significant association was detected between breastfeeding initiation and duration and the occurrence of ASD, among children in Sulaymaniyah, Kurdistan Region of Iraq. Although some previous studies have detected potential protective effects, the evidence remains inconsistent. These findings underscore the multifactorial nature of ASD and highlight the need for large-scale, prospective studies to further investigate potential causal relationships and address existing gaps in the literature.

Acknowledgments

The authors acknowledge Dr. Mohammed Ibrahim Mohialdeen Gubari, PhD (College of Medicine, University of Sulaimani), for his methodological advice during questionnaire development and sample size estimation. The authors also acknowledge the Autism Detection Committee at the Directorate of Health in Sulaymaniyah, the Psychiatry Department at Dr. Jamal's Pediatric Teaching Hospital, and Papulakani Kurdistan Center for Life Skills for their administrative and clinical support during participant recruitment. The authors further acknowledge the parents and guardians of the participating children for their cooperation in this study.

Conflict of Interest Statement

The authors declare no conflicts of interest related to this manuscript.

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