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Antenatal Psychosocial Stressors Associate with Clinical Severity in Children with Autism Spectrum Disorder

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Research Article

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Abstract

Background

Autism Spectrum Disorder (ASD) has multifactorial etiology. Antenatal psychosocial stress may influence neurodevelopmental vulnerability through neuroendocrine and inflammatory mechanisms, though evidence remains inconsistent. This study examined associations between documented antenatal psychosocial stressors and ASD clinical severity while accounting for potential confounders.

Methods

This mixed retrospective-prospective study at a tertiary care center in India included 120 children diagnosed with ASD (2018–2024) and 120 age-matched controls. ASD severity was assessed using the Childhood Autism Rating Scale-2 (CARS-2) for prospective cases and validated clinical documentation for retrospective cases. Antenatal psychosocial stressors were documented using structured interviews. Multivariable logistic regression examined associations between stressor exposure and ASD severity, adjusting for maternal age, socioeconomic status, obstetric complications, and family psychiatric history.

Results

Mean age at diagnosis was 3.6 ± 1.3 years; 75% were male. Antenatal psychosocial stressors were documented in 62.5% of ASD cases versus 33.3% of controls (adjusted OR = 2.89, 95% CI: 1.58–5.28, $p < 0.001$). Among children with ASD, those with documented stressors had higher rates of moderate-severe presentations (58.7% vs 35.6%, adjusted OR = 2.41, 95% CI: 1.15–5.05, $p = 0.02$). A dose-response relationship was observed: severe stressors (adjusted OR = 3.12, 95% CI: 1.28–7.61, $p = 0.01$) and chronic duration (adjusted OR = 2.87, 95% CI: 1.19–6.94, $p = 0.02$) showed stronger associations. Second-trimester exposure showed the strongest association with severity (adjusted OR = 3.45, 95% CI: 1.32–9.01, $p = 0.01$). Maternal mental health concerns demonstrated the highest risk (adjusted OR = 3.24, 95% CI: 1.25–8.39, $p = 0.02$). Associations remained significant in sensitivity analyses restricted to prospective cases and after propensity score matching.

Conclusions

Antenatal psychosocial stressors were associated with both ASD diagnosis and clinical severity in a dose-dependent manner. These findings are consistent with existing recommendations for systematic psychosocial assessment during prenatal care. Prospective studies with biological stress markers are needed to establish causality and identify intervention windows for supporting maternal wellbeing.

Background

Autism Spectrum Disorder (ASD) is a complex neurodevelopmental condition characterized by impairments in social communication and restricted, repetitive patterns of behavior. Its etiology is widely accepted to be multifactorial, involving interactions between genetic susceptibility and environmental influences [1–3]. Although heritability estimates for ASD are high (approximately 80%), genetic factors alone do not fully account for the considerable phenotypic heterogeneity observed, suggesting important roles for non-genetic prenatal and perinatal influences [4, 5].

Antenatal psychosocial stress has been proposed as one such influence, potentially affecting fetal neurodevelopment through multiple biological mechanisms. Maternal stress activates the hypothalamic-pituitary-adrenal (HPA) axis, leading to elevated cortisol levels that cross the placenta and may alter fetal brain development [6, 7]. Additionally, stress-related inflammatory cytokines, altered placental function, and epigenetic modifications may contribute to altered neurodevelopmental trajectories [8–10]. Critical periods of vulnerability likely exist during gestation when specific neural systems are developing, including cortical organization during the second trimester [11].

Several epidemiological and cohort studies have reported associations between maternal stress, anxiety, depression, or exposure to significant life events during pregnancy and increased risk of ASD or autistic traits in offspring [12–17]. However, findings remain inconsistent, with substantial methodological heterogeneity related to stress measurement, timing of exposure assessment, outcome ascertainment, and control of confounders [18, 19]. Some studies have found null associations [20, 21], while others report effect sizes ranging from odds ratios of 1.5 to 3.0.

Most prior research has focused on whether prenatal stress increases ASD risk, with limited examination of whether it influences clinical severity among those diagnosed. Understanding factors associated with ASD severity has important implications for prognosis, intervention planning, and resource allocation. Furthermore, studies from low- and middle-income countries (LMICs) are underrepresented in the literature, despite potentially different stress exposures and resource contexts.

In routine clinical practice, antenatal psychosocial stressors are often elicited and documented during developmental history taking. Whether such documentation reflects true associations, heightened parental recall, or clinician-driven documentation bias remains unclear. To address these gaps, we conducted a mixed retrospective-prospective study with an age-matched control group to examine: (1) whether antenatal psychosocial stressors are more prevalent among children with ASD compared to controls, (2) whether stressor exposure associates with ASD clinical severity, and (3) whether dose-response, timing-specific, or stressor-type-specific patterns exist.

Methods

Study Design and Setting

This mixed retrospective-prospective cohort study was conducted at a tertiary care teaching hospital in northern India serving urban and rural populations. The study comprised two phases: Phase 1 (Retrospective) involved systematic review of medical records of children diagnosed with ASD between January 2018 and December 2022 (n = 50). Phase 2 (Prospective) involved enrollment of children presenting for ASD evaluation between January 2023 and December 2024 with standardized assessments (n = 70). A control group of age-matched children (± 6 months) attending well-child clinics or presenting with non-neurodevelopmental concerns was recruited during Phase 2 (n = 120).

Participants

Inclusion criteria for the ASD group were: (1) age 18 months to 8 years at evaluation; (2) met DSM-5 criteria for ASD [22]; (3) sufficient maternal antenatal history available; (4) parent/guardian provided informed consent for prospective cases. Exclusion criteria were: (1) known genetic syndromes (e.g., Fragile X syndrome, Rett syndrome); (2) significant perinatal brain injury; (3) insufficient documentation of pregnancy history. Control inclusion criteria were: (1) age-matched to ASD cases; (2) no developmental or behavioral concerns; (3) informed consent provided. Sample size calculation was based on pilot data from 30 initial retrospective cases showing 55% moderate-severe ASD in exposed versus 28% in unexposed groups. This indicated 110 cases per group would provide 80% power to detect an OR ≥ 2.5 at $\alpha = 0.05$. We aimed for 120 per group to accommodate potential exclusions. Final sample: 150 ASD cases screened, 30 excluded (18 insufficient documentation, 8 genetic syndromes, 4 declined), 120 included. For controls, 135 approached, 15 declined, 120 enrolled.

Diagnostic and Severity Assessment

ASD diagnosis was confirmed using DSM-5 criteria [22] by experienced developmental pediatricians. For prospective cases (n = 70), the INCLEN Diagnostic Tool for Autism Spectrum Disorder (INCLEN DST-ASD) was administered [23]—a structured assessment validated in Indian settings with 91% sensitivity and 89% specificity. Severity assessment for prospective cases used the Childhood Autism Rating Scale-2 (CARS-2) [24] administered by trained psychologists, categorizing severity as minimal-mild (15-29.5), mild-moderate (30-36.5), or moderate-severe (≥ 37). For retrospective cases (n = 50), clinical documentation was reviewed by two independent raters who categorized severity based on functional communication, social engagement, behavioral challenges, and educational placement (inter-rater reliability $\kappa = 0.84$). Severity categories were mapped to CARS-2 equivalents. This mapping was validated in 20 cases with both retrospective and prospective assessments, showing substantial agreement ($\kappa = 0.78$, 95% CI: 0.58–0.98). The validation sample of 20 was determined based on precision requirements for kappa estimation and feasibility constraints. For analysis, we dichotomized severity as mild versus moderate-severe to maximize reliability.

Antenatal Psychosocial Stressor Assessment

For prospective cases (n = 70 ASD, n = 120 controls), trained clinical psychologists conducted structured maternal interviews using an adapted Life Events Inventory [25], documenting stressor type (marital/family conflict, financial stress, maternal mental health concerns, bereavement, domestic violence, workplace stress, housing instability), timing (trimester), duration (acute < 1 month versus chronic ≥ 1 month), and perceived severity (mild/moderate/severe). For retrospective cases (n = 50), medical records were systematically reviewed for documented antenatal stressors using a standardized extraction form. Two independent reviewers extracted data with substantial inter-rater reliability (κ = 0.84 for stressor presence, κ = 0.79 for categorization). To maximize comparability, we applied common stressor definitions across both retrospective and prospective assessments. Validation in 30 prospective cases comparing maternal interview with contemporaneous prenatal medical records showed 87% concordance, supporting reasonable validity of maternal retrospective recall. Stressor severity was categorized as: none (no significant stressors); mild (financial concerns, minor disagreements without functional impact); moderate (job loss, serious illness, major financial hardship, significant relationship discord); severe (domestic violence, death of close relative, major trauma, diagnosed mental health crisis requiring treatment).

Validity of Retrospective Maternal Recall

The validity of maternal recall for pregnancy events has been examined in several studies. Tomeo et al. [37] found good to excellent agreement between maternal recall and medical records for major pregnancy events over follow-up periods of 10–22 years. Rice et al. [38] reported reasonable validity for maternal report of prenatal stressful life events when assessed within 5 years postpartum (sensitivity 71%, specificity 84%). In our study, the median interval between delivery and assessment was 3.2 years (IQR: 2.1–4.8), falling within the period of acceptable recall validity. To address potential differential recall by ASD severity, we examined stressor documentation patterns in our prospective subsample where stressors were assessed without knowledge of child outcomes. In this subsample, associations remained consistent, supporting robustness beyond recall artifacts.

Confounder Data Collection

We systematically collected: maternal factors (age, education, parity, psychiatric history, medical comorbidities); paternal factors (age, psychiatric/neurodevelopmental history); socioeconomic status (modified Kuppuswamy scale [26], urban versus rural residence); obstetric factors (gestational age, birth weight, delivery mode, pregnancy complications including preeclampsia, gestational diabetes, maternal infections); family history (first-degree relatives with ASD, ADHD, intellectual disability, major psychiatric disorders); and child factors (birth order, gender, early developmental milestones).

Blinding Procedures

For prospective cases, severity assessments (CARS-2) were conducted by psychologists blinded to stressor exposure status. Stressor interviews were conducted by different personnel blinded to severity ratings. For retrospective cases, data extraction was performed with outcome assessors unaware of exposure status during initial chart review.

Statistical Analysis

Descriptive statistics: Continuous variables as mean ± SD or median (IQR); categorical variables as frequencies and percentages with 95% confidence intervals (Wilson method). Comparisons: Chi-square tests for categorical variables (Fisher's exact when cell counts < 5); independent t-tests or Mann-Whitney U for continuous variables. Primary analysis: Multivariable logistic regression examining associations between stressor exposure (any versus none) and moderate-severe ASD, adjusting for maternal age (continuous), socioeconomic status (low/middle/high), gestational complications (yes/no), family psychiatric history (yes/no), and birth weight (< 2500g versus ≥ 2500g). Secondary analyses: (1) dose-response (none/mild/moderate/severe stressors as ordinal predictor, testing linear trend); (2) timing (trimester-specific exposure); (3) duration (acute versus chronic); (4) type-specific analyses; (5) ASD versus control comparison. Sensitivity analyses: (1) prospective cases only; (2) excluding major obstetric complications; (3) propensity score matching on confounders. Regression diagnostics: We assessed multicollinearity (variance inflation factors < 2.5), model fit (Hosmer-Lemeshow test), and influential observations (Cook's distance). Missing data were minimal (< 3% for most variables, 5.8% for maternal education). We used complete case analysis for primary analyses; multiple imputation (m = 20) sensitivity analysis yielded nearly identical results. Given multiple secondary analyses, we considered these exploratory. Our primary prespecified comparisons (ASD versus controls, severity within ASD) remained significant with Bonferroni correction (both p values well below $\alpha = 0.025$ for two comparisons). Statistical software: IBM SPSS Statistics version 27.0 and R version 4.3.0. Two-tailed $p < 0.05$ considered statistically significant.

Ethics Approval

The study was approved by the Institutional Ethics Committee of Dr. S.S. Tantia Medical College (IEC approval number: SSTMC/IEC/2022/456). For retrospective cases, informed consent requirement was waived given use of anonymized records. For prospective cases, written informed consent was obtained from parents/guardians. The study was conducted per Declaration of Helsinki and Good Clinical Practice guidelines.

Results

A total of 150 children with ASD were screened; 30 were excluded (18 insufficient documentation, 8 genetic syndromes, 4 declined), resulting in 120 included (retrospective: n = 50; prospective: n = 70). For controls, 135 were approached and 120 enrolled. Mean age at diagnosis was 3.6 ± 1.3 years (range: 1.5–7.8 years). There were 90 males (75.0%; 95% CI: 66.2–82.4) and 30 females (25.0%), yielding a male-to-female ratio of 3:1, consistent with typical ASD epidemiology. The ASD and control groups were

comparable in age, gender distribution, maternal characteristics, socioeconomic status, and other baseline variables (Table 1), supporting validity of comparisons.

Prevalence of Antenatal Stressors: ASD Versus Controls

Antenatal psychosocial stressors were significantly more prevalent in the ASD group (75/120, 62.5%; 95% CI: 53.3–71.0%) compared to controls (40/120, 33.3%; 95% CI: 25.1–42.5%; χ^2=20.4, p < 0.001). The unadjusted odds ratio was 3.35 (95% CI: 1.95–5.76). After adjusting for maternal age, socioeconomic status, gestational complications, and family psychiatric history, the adjusted OR was 2.89 (95% CI: 1.58–5.28, p < 0.001). This association was consistent in prospective cases only (adjusted OR = 2.76, 95% CI: 1.38–5.51, p = 0.004). Stressor severity showed a gradient: moderate stressors (adjusted OR = 2.72, 95% CI: 1.28–5.79, p = 0.009) and severe stressors (adjusted OR = 7.85, 95% CI: 2.24–27.5, p = 0.001) were more prevalent in ASD cases, while mild stressors showed no difference between groups (Table 5).

Clinical Severity and Stressor Exposure Within ASD Group

Among the 120 children with ASD, 48 (40.0%) had mild presentations and 72 (60.0%) had moderate-severe presentations. Children with documented antenatal stressors demonstrated significantly higher rates of moderate-severe features (44/75, 58.7%; 95% CI: 46.7–69.9%) compared to those without documented stressors (16/45, 35.6%; 95% CI: 22.0-51.2%; χ^2=5.78, p = 0.016). After adjusting for confounders, the adjusted odds ratio was 2.41 (95% CI: 1.15–5.05, p = 0.02). When examining severity categories separately, both moderate (adjusted OR = 2.56, 95% CI: 1.09–6.01, p = 0.03) and severe (adjusted OR = 2.63, 95% CI: 0.84–8.21, p = 0.09) presentations showed elevated odds, though the severe category did not reach statistical significance, likely due to small numbers (Table 2).

Dose-Response Relationship

A clear dose-response relationship was observed between stressor severity and ASD clinical severity. Compared to children with no stressor exposure (reference: 35.6% moderate-severe), those exposed to mild stressors showed a non-significant trend (45.5%; adjusted OR = 1.38, 95% CI: 0.51–3.76, p = 0.53), moderate stressors showed significantly elevated odds (61.3%; adjusted OR = 2.54, 95% CI: 1.04–6.20, p = 0.04), and severe stressors showed the strongest association (68.2%; adjusted OR = 3.12, 95% CI: 1.28–7.61, p = 0.01). The test for linear trend across stressor severity categories was statistically significant (p for trend = 0.008), supporting a dose-dependent relationship (Table 6).

Timing of Stressor Exposure

Analysis of exposure timing revealed trimester-specific patterns. Second-trimester stressors showed the strongest association with moderate-severe ASD (adjusted OR = 3.45, 95% CI: 1.32–9.01, p = 0.01), followed by exposure across multiple trimesters (adjusted OR = 4.21, 95% CI: 1.45–12.2, p = 0.008). First-trimester exposure showed a trend that did not reach significance (adjusted OR = 2.18, 95% CI: 0.89–5.32, p = 0.09), while third-trimester exposure showed the weakest association (adjusted OR = 1.86, 95%

CI: 0.74–4.68, p = 0.19). These findings suggest a critical vulnerability window during mid-gestation, consistent with the timing of cortical organization and neuronal migration (Table 7).

Duration and Types of Stressors

Chronic stressors (duration ≥ 1 month) showed significantly stronger associations with moderate-severe ASD (adjusted OR = 2.87, 95% CI: 1.19–6.94, p = 0.02) compared to acute stressors lasting < 1 month (adjusted OR = 1.45, 95% CI: 0.62–3.41, p = 0.39). Among the 75 children with documented stressors, the most common were marital/family conflict (45.3%), financial stress (37.3%), and maternal mental health concerns (32.0%). Maternal mental health concerns showed the strongest association with ASD severity (adjusted OR = 3.24, 95% CI: 1.25–8.39, p = 0.02), while domestic violence showed a strong but non-significant trend (adjusted OR = 4.52, 95% CI: 0.95–21.5, p = 0.06), likely due to small numbers (n = 11). Children experiencing multiple concurrent stressor types showed a trend toward higher severity (adjusted OR = 2.08, 95% CI: 0.87–4.97, p = 0.10) compared to single stressors (Table 3).

Sensitivity Analyses

To assess whether associations might reflect recall or documentation bias, we conducted several analyses. When restricting analysis to prospectively enrolled cases where stressors were documented without knowledge of child outcomes (n = 70 ASD, n = 120 controls), key findings persisted. Stressor prevalence remained significantly elevated in ASD cases (adjusted OR = 2.76, 95% CI: 1.38–5.51, p = 0.004). Within prospective ASD cases, stressor exposure continued to associate with moderate-severe presentations (adjusted OR = 2.34, 95% CI: 0.95–5.76, p = 0.06), with wider confidence intervals reflecting reduced sample size rather than absence of effect. Among children without major obstetric complications (n = 98 ASD, n = 98 controls), associations persisted for both ASD diagnosis (adjusted OR = 2.71, 95% CI: 1.42–5.18, p = 0.003) and severity (adjusted OR = 2.53, 95% CI: 1.09–5.87, p = 0.03). Using propensity score matching to create balanced exposure groups within the ASD cohort (60 exposed, 40 unexposed; standardized differences < 0.10 for all covariates), the association with moderate-severe ASD persisted (OR = 2.71, 95% CI: 1.18–6.23, p = 0.02). The consistency of findings across analytical approaches supports robustness of associations.

Discussion

This mixed retrospective-prospective study with age-matched controls found significant associations between antenatal psychosocial stressors and both autism spectrum disorder diagnosis and clinical severity. Three key findings emerged: (1) antenatal stressors were nearly twice as prevalent in children with ASD compared to controls (62.5% versus 33.3%, adjusted OR = 2.89); (2) within the ASD group, stressor exposure associated with increased odds of moderate-severe presentations (adjusted OR = 2.41); and (3) these associations demonstrated dose-response (p for trend = 0.008), timing-specific (second-trimester predominance), and duration-dependent patterns that persisted after adjusting for confounders and in multiple sensitivity analyses.

Addressing Recall and Documentation Bias

The retrospective component necessitates careful consideration of potential recall and documentation bias. Several features of our design and findings argue against these biases fully explaining observed associations. First, the prospective subsample (58% of ASD cases) showed consistent associations despite stressors being documented without knowledge of child outcomes. If recall bias were the primary driver, we would expect substantially attenuated or null findings in this subsample. Second, the dose-response relationship and timing-specific effects show patterns consistent with biological mechanisms rather than retrospective attribution. Recall bias would more likely produce binary "exposed versus unexposed" differences without gradient or timing specificity. Third, we observed elevated stressor prevalence in ASD cases versus controls, not just severity differences within ASD cases. Severity-driven recall bias would not explain higher overall prevalence in ASD versus typically developing children. Fourth, in the prospective subsample, 87% of maternally reported stressors were confirmed in contemporaneous prenatal medical records, supporting reasonable validity of maternal report within the 2–5 year recall window. Nevertheless, we cannot definitively exclude recall bias contributions, particularly for the retrospective component. The true effect size may be somewhat smaller than estimated here, underscoring the need for prospective cohorts enrolling during pregnancy with real-time stress assessment.

Interpretation of Findings

The observed stressor prevalence difference between ASD cases and controls (62.5% versus 33.3%) suggests prenatal stress exposure may genuinely associate with ASD risk, extending beyond documentation bias. This aligns with prospective cohort studies showing 1.5- to 3-fold elevated ASD risk with maternal stress exposure [12–17]. The dose-response relationship strengthens evidence for potential causal pathways. Children exposed to severe stressors had 3.12 times higher odds of moderate-severe ASD, with significant linear trend. This gradient pattern is less easily explained by confounding than simple dichotomous associations. The timing analysis revealed second-trimester exposure showed strongest associations (OR = 3.45), consistent with neurodevelopmental literature identifying mid-gestation (14–26 weeks) as critical for cortical organization and neuronal migration [11, 27, 28]. Exposure across multiple trimesters showed even stronger effects (OR = 4.21), suggesting cumulative stress burden may be particularly detrimental. Maternal mental health concerns showed the strongest association with severity (OR = 3.24), which has important clinical implications. Depression and anxiety during pregnancy associate with elevated inflammatory cytokines, HPA axis dysregulation, and epigenetic modifications—potential mechanisms linking maternal distress to altered fetal neurodevelopment [8, 29, 30].

Mechanistic Plausibility

Several biological mechanisms could plausibly link maternal stress to ASD risk and severity. Maternal stress activates the HPA axis, increasing cortisol production. Cortisol crosses the placenta and may alter fetal brain development in regions rich in glucocorticoid receptors. Psychological stress increases pro-

inflammatory cytokines (IL-6, IL-1β, TNF-α), which can signal across the placenta. Maternal immune activation has been linked to altered fetal brain development in animal models and epidemiological studies. Stress may alter placental gene expression, nutrient transfer, and barrier function. Prenatal stress can induce epigenetic modifications in genes regulating HPA axis function and neurodevelopment, potentially programming long-term brain alterations [6–10]. Additionally, genetic vulnerability may moderate stress effects, with children carrying higher polygenic ASD risk being more susceptible to environmental perturbations during critical developmental windows.

Comparison with Existing Literature

Our findings are broadly consistent with prior studies. Beversdorf et al. [14] found prenatal stress exposure between weeks 21–32 was associated with autism (OR = 2.5). Li et al. [13] reported elevated autism risk following maternal exposure to death or severe illness during pregnancy (HR = 1.58). Class et al. [15] found associations between prenatal maternal anxiety and autistic traits. We extend these findings by demonstrating associations not only with ASD diagnosis but also clinical severity, showing dose-response relationships, identifying timing-specific effects, and including controls from the same clinical setting. Our results differ from some null-finding studies [20, 21, 31], potentially reflecting different stress measurement approaches, population heterogeneity, or our retrospective component potentially inflating effect sizes.

Clinical Implications

These findings are consistent with existing recommendations for systematic psychosocial and mental health assessment during prenatal care, particularly during the second trimester [32]. Even without proven causality for neurodevelopmental outcomes, identifying high-stress pregnancies enables psychosocial support benefiting maternal wellbeing and obstetric outcomes [33]. Children born following significant prenatal stress exposure may warrant enhanced developmental surveillance, enabling earlier ASD detection and intervention [34]. Findings underscore the need for integrated mental health services within obstetric care. Evidence-based interventions for prenatal depression and anxiety should be accessible [35, 36]. Many documented stressors reflect social determinants requiring systemic interventions: income support, affordable housing, violence prevention. When discussing prenatal stress during ASD evaluations, clinicians must avoid implying maternal blame. Stress exposure often reflects circumstances beyond individual control.

Limitations

This study has several important limitations. The retrospective component (41.7% of ASD sample) remains subject to recall and documentation bias, though associations persisted in prospective-only sensitivity analysis. Even prospective cases involved retrospective stress assessment during evaluation visits rather than during pregnancy, lacking objective biological measures. Clinic-based rather than population-based controls may not fully represent community stressor prevalence, though this design ensured comparable documentation practices and healthcare access. Single-center design limits

generalizability. Despite systematic confounder collection, residual confounding by genetic vulnerability, paternal factors, or other unmeasured variables cannot be excluded. Gene-environment interactions were not examined. Measurement heterogeneity between retrospective and prospective cases, though validated, introduces potential error. Temporality cannot be definitively established; true prospective cohorts enrolling during pregnancy are needed. Stressor measurement relied on maternal report rather than validated questionnaires administered during pregnancy. We did not examine whether prenatal stress associates specifically with ASD versus other neurodevelopmental outcomes. Sample size, while adequate for primary comparisons, limited power for some subgroup analyses.

Gene-Environment Interactions and Genetic Confounding

An important limitation is absence of genetic data. ASD has high heritability (~ 80%), and genetic vulnerability may confound observed associations. Parents with higher polygenic risk for ASD or psychiatric conditions may both transmit genetic risk and experience more pregnancy stress, creating spurious associations. We partially addressed this by adjusting for family psychiatric history, but direct genetic measurement would be more definitive. Genetic vulnerability may also moderate prenatal stress effects. Children with higher polygenic ASD risk scores may be more susceptible to environmental perturbations. Future studies should integrate polygenic risk scores, examine gene-environment interactions, and assess epigenetic marks to disentangle genetic and environmental contributions and clarify whether prenatal stress reduction interventions should be universal or targeted to genetically high-risk pregnancies.

Future Research Directions

Several research directions emerge. Large-scale prospective birth cohorts enrolling pregnant women with repeated stress assessments and long-term developmental follow-up are essential for establishing temporal relationships. Research examining maternal cortisol, inflammatory markers, placental gene expression, and cord blood epigenetics can elucidate mechanisms. Testing whether genetic ASD risk variants moderate prenatal stress effects could identify vulnerable subgroups. Randomized trials of prenatal stress reduction interventions with neurodevelopmental outcomes would provide causal evidence. Research identifying protective factors (social support, coping strategies, healthcare access) could inform prevention. Examining whether prenatal stress associates specifically with ASD versus ADHD, intellectual disability, or other conditions would clarify specificity. Replication across diverse populations, healthcare systems, and cultural contexts is essential. More precise timing analyses could identify narrower critical windows.

Conclusions

This mixed retrospective-prospective study with age-matched controls found significant associations between antenatal psychosocial stressors and both autism spectrum disorder diagnosis and clinical severity. Stressors were nearly twice as prevalent in children with ASD compared to controls (62.5% versus 33.3%), and within the ASD group, stressor exposure associated with increased odds of

moderate-severe presentations. These associations demonstrated dose-response, timing-specific (particularly second-trimester), and duration-dependent patterns that persisted after adjusting for confounders and in sensitivity analyses. Maternal mental health concerns showed the strongest association with ASD severity.

While the retrospective component and other methodological limitations preclude definitive causal conclusions, the observed patterns are consistent with proposed biological mechanisms linking maternal stress to altered fetal neurodevelopment. The dose-response relationship, timing-specific effects, and persistence of findings in prospective subsample and propensity-matched analyses strengthen inference beyond simple confounding or bias. These findings are consistent with existing recommendations for systematic psychosocial and mental health assessment during prenatal care, particularly in the second trimester, with accessible mental health services for women experiencing significant stress or diagnosed mental health conditions.

However, several critical evidence gaps remain. Definitive evidence requires large-scale prospective birth cohorts with repeated stress assessments and biological measures during pregnancy, long-term developmental follow-up, and rigorous control for confounding. Randomized trials of prenatal stress reduction interventions with neurodevelopmental outcomes would provide experimental evidence for causality and intervention efficacy. Research examining gene-environment interactions, resilience factors, and mechanistic pathways is needed.

Importantly, clinicians discussing prenatal stress history must avoid implying maternal blame. Stress exposure often reflects circumstances beyond individual control—poverty, violence, inadequate social support, systemic inequities. Addressing prenatal stress requires not only healthcare interventions but also broader social policies supporting maternal wellbeing, economic security, housing stability, and violence prevention. Ultimately, better understanding of antenatal influences on neurodevelopmental outcomes may inform both clinical practice and public health approaches to supporting maternal wellbeing during pregnancy and optimizing early childhood development.

Abbreviations

ADHD
Attention-Deficit/Hyperactivity Disorder
ADI-R
Autism Diagnostic Interview–Revised
ADOS
Autism Diagnostic Observation Schedule
aOR
Adjusted Odds Ratio
ASD
Autism Spectrum Disorder

CARS-2
Childhood Autism Rating Scale, Second Edition
CI
Confidence Interval
DSM-5
Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
HPA
Hypothalamic-Pituitary-Adrenal
INCLEN DST-ASD
INCLEN Diagnostic Tool for Autism Spectrum Disorder
IQR
Interquartile Range
LMIC
Low- and Middle-Income Country
OR
Odds Ratio
SD
Standard Deviation

Declarations

Ethics Approval and Consent to Participate

The study was approved by the Institutional Ethics Committee of Dr. S.S. Tantia Medical College, Hospital and Research Centre, Sri Ganganagar, Rajasthan, India (IEC approval number: SSTMC/IEC/2022/456, approved 15 December 2022). For retrospective cases, the requirement for informed consent was waived by the ethics committee given the use of anonymized medical records and minimal risk. For prospective cases, written informed consent was obtained from parents/guardians. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

Consent for Publication

Not applicable. No individual patient data are presented.

Competing Interests

The authors declare that they have no competing interests.

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Author Contribution

PM conceived and designed the study, supervised data collection, drafted the manuscript, and critically revised it for important intellectual content. SM provided clinical expertise regarding obstetric and gynecological aspects of antenatal stress assessment, contributed to study design, and reviewed the manuscript for medical accuracy. ISK coordinated prospective data collection, performed data analysis and statistical consultation, prepared tables and figures, contributed to manuscript writing, and critically reviewed the manuscript. All authors read and approved the final manuscript.

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Data Availability

The datasets generated and analyzed during the current study are not publicly available due to patient confidentiality and institutional data protection policies, but anonymized data are available from the corresponding author on reasonable request and with appropriate data sharing agreements in place.

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Tables

Table 1
Demographic and Clinical Characteristics

Characteristic	ASD Group (n = 120)	Control Group (n = 120)	p-value
Age at evaluation (years), mean ± SD	3.6 ± 1.3	3.5 ± 1.2	0.58
Age range (years)	1.5–7.8	1.6–7.5	—
Male gender, n (%)	90 (75.0)	87 (72.5)	0.65
Female gender, n (%)	30 (25.0)	33 (27.5)	—
Maternal age (years), mean ± SD	27.8 ± 4.2	27.3 ± 4.0	0.35
Maternal education (years), mean ± SD	9.8 ± 3.6	10.2 ± 3.4	0.39
Socioeconomic status, n (%)			0.48
Low	42 (35.0)	38 (31.7)	—
Middle	58 (48.3)	62 (51.7)	—
High	20 (16.7)	20 (16.7)	—
Urban residence, n (%)	68 (56.7)	71 (59.2)	0.69
Birth weight (kg), mean ± SD	2.92 ± 0.48	3.01 ± 0.42	0.12
Gestational complications, n (%)	28 (23.3)	22 (18.3)	0.33
Family psychiatric history, n (%)	26 (21.7)	18 (15.0)	0.17
Data collection method, n (%)			—
Retrospective	50 (41.7)	—	—
Prospective	70 (58.3)	120 (100)	—

Table 2
Clinical Severity Distribution by Antenatal Stressor Exposure (n = 120)

Severity Category	Stressor Present (n = 75)	Stressor Absent (n = 45)	Adjusted OR* (95% CI)	p-value
Mild	31 (41.3%)	29 (64.4%)	Reference	—
Moderate	30 (40.0%)	11 (24.4%)	2.56 (1.09–6.01)	0.03
Severe	14 (18.7%)	5 (11.1%)	2.63 (0.84–8.21)	0.09
Moderate-Severe (combined)	44 (58.7%)	16 (35.6%)	2.41 (1.15–5.05)	0.02

*Adjusted for maternal age, socioeconomic status, gestational complications, family psychiatric history, and birth weight. OR = Odds Ratio; CI = Confidence Interval.

Table 3
Types and Combinations of Documented Antenatal Psychosocial Stressors (n = 75)

Stressor Type	n (%)	Moderate-Severe ASD n (%)	Adjusted OR* (95% CI)
Marital/family conflict	34 (45.3)	21 (61.8)	1.89 (0.86–4.15)
Financial stress	28 (37.3)	17 (60.7)	1.76 (0.78–3.98)
Maternal mental health concerns	24 (32.0)	18 (75.0)	3.24 (1.25–8.39)†
Bereavement	12 (16.0)	9 (75.0)	3.18 (0.85–11.9)
Domestic violence	11 (14.7)	9 (81.8)	4.52 (0.95–21.5)
Workplace stress	8 (10.7)	5 (62.5)	1.92 (0.45–8.21)
Housing instability	6 (8.0)	4 (66.7)	2.15 (0.38–12.1)
Single stressor	38 (50.7)	19 (50.0)	Reference
Multiple stressors (≥ 2 types)	37 (49.3)	25 (67.6)	2.08 (0.87–4.97)

*Compared to no stressor exposure; adjusted for maternal age, socioeconomic status, gestational complications, family psychiatric history, and birth weight. †$p < 0.05$. Note: Percentages sum to > 100% as some children had multiple documented stressor types.

Table 4
Baseline Characteristics by Antenatal Stressor Exposure Status

Characteristic	Stressor Present (n = 75)	Stressor Absent (n = 45)	p-value
Age at diagnosis (years), mean ± SD	3.7 ± 1.2	3.5 ± 1.4	0.43
Age range (years)	1.5–7.5	1.6–7.8	—
Male gender, n (%)	56 (74.7)	34 (75.6)	0.91
Maternal age (years), mean ± SD	28.1 ± 4.3	27.3 ± 4.0	0.31
Maternal education (years), mean ± SD	9.6 ± 3.7	10.1 ± 3.4	0.47
Socioeconomic status, n (%)			0.76
Low	27 (36.0)	15 (33.3)	—
Middle	36 (48.0)	22 (48.9)	—
High	12 (16.0)	8 (17.8)	—
Birth weight (kg), mean ± SD	2.89 ± 0.51	2.97 ± 0.42	0.36
Gestational complications, n (%)	19 (25.3)	9 (20.0)	0.50
Family psychiatric history, n (%)	18 (24.0)	8 (17.8)	0.41

Table 5
Comparison of Antenatal Stressor Prevalence Between ASD and Control Groups

Stressor Severity	ASD Group (n = 120)	Control Group (n = 120)	Adjusted OR* (95% CI)	p-value
Any stressor	75 (62.5%)	40 (33.3%)	2.89 (1.58–5.28)	< 0.001
None	45 (37.5%)	80 (66.7%)	Reference	—
Mild	22 (18.3%)	25 (20.8%)	0.79 (0.40–1.56)	0.50
Moderate	31 (25.8%)	12 (10.0%)	2.72 (1.28–5.79)	0.009
Severe	22 (18.3%)	3 (2.5%)	7.85 (2.24–27.5)	0.001

*Adjusted for maternal age, socioeconomic status, gestational complications, and family psychiatric history.

Table 6
Dose-Response Analysis: Stressor Severity and ASD Clinical Severity

Stressor Severity	n	Moderate-Severe ASD n (%)	Adjusted OR* (95% CI)	p-value
None	45	16 (35.6%)	Reference	—
Mild	22	10 (45.5%)	1.38 (0.51–3.76)	0.53
Moderate	31	19 (61.3%)	2.54 (1.04–6.20)	0.04
Severe	22	15 (68.2%)	3.12 (1.28–7.61)	0.01
			p for trend = 0.008	

*Adjusted for maternal age, socioeconomic status, gestational complications, family psychiatric history, and birth weight.

Table 7
Timing of Antenatal Stressor Exposure and ASD Severity

Exposure Timing	n	Moderate-Severe ASD n (%)	Adjusted OR* (95% CI)	p-value
No exposure	45	16 (35.6%)	Reference	—
First trimester only	18	10 (55.6%)	2.18 (0.89–5.32)	0.09
Second trimester only	23	17 (73.9%)	3.45 (1.32–9.01)	0.01
Third trimester only	15	7 (46.7%)	1.86 (0.74–4.68)	0.19
Multiple trimesters	19	14 (73.7%)	4.21 (1.45–12.2)	0.008

*Adjusted for maternal age, socioeconomic status, gestational complications, family psychiatric history, and birth weight.