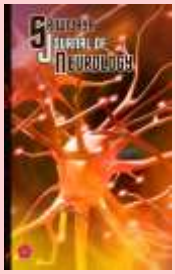

e-ISSN: 2987-1425

Sriwijaya Journal of Neurology (SJN)

Journal website: https://phlox.or.id/index.php/sjn

Prenatal Exposure to Maternal Stress and Neurodevelopmental Outcomes in Children: A Longitudinal Study with Epigenetic Analysis in Jakarta, Indonesia

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ARTICLE INFO

Keywords:
Child development
DNA methylation
Epigenetics
Neurodevelopment
Prenatal stress

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All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.59345/sjn.v1i2.81

A B S T R A C T

Introduction: Prenatal exposure to maternal stress has been identified as a potential risk factor for adverse neurodevelopmental outcomes in children. This study aimed to investigate the association between prenatal maternal stress and neurodevelopmental outcomes in children in Jakarta, Indonesia, and to explore the potential mediating role of epigenetic modifications. **Methods:** A longitudinal cohort study was conducted involving 300 pregnant women recruited from antenatal clinics in Jakarta. Maternal stress was assessed during the second trimester of pregnancy using the Perceived Stress Scale (PSS). Neurodevelopmental outcomes in children were evaluated at 12 and 24 months of age using the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III). Epigenetic analysis of cord blood DNA methylation was performed using the Illumina Infinium MethylationEPIC BeadChip. **Results:** Higher maternal stress scores during pregnancy were significantly associated with lower cognitive, language, and motor scores in children at 12 and 24 months of age. Epigenetic analysis revealed differential methylation patterns in genes related to neurodevelopment in children exposed to high prenatal maternal stress. Mediation analysis indicated that DNA methylation partially mediated the association between prenatal maternal stress and neurodevelopmental outcomes. **Conclusion:** Prenatal exposure to maternal stress is associated with adverse neurodevelopmental outcomes in children, and epigenetic modifications may play a mediating role in this relationship. These findings highlight the importance of addressing maternal stress during pregnancy to promote optimal child neurodevelopment.

1. Introduction

The period of prenatal development is a critical window of vulnerability for the developing fetus, during which exposure to adverse environmental factors can have profound and long-lasting effects on the trajectory of child development. These early experiences can shape the structure and function of the developing brain, influencing a wide range of outcomes, including cognitive abilities, emotional regulation, and behavioral patterns. Among the various environmental factors that can impact fetal development, maternal stress during pregnancy has emerged as a significant risk factor for adverse neurodevelopmental outcomes in children. Maternal stress during pregnancy encompasses a complex interplay of biological, psychological, and social factors

that can disrupt the delicate balance of the intrauterine environment. When a pregnant woman experiences stress, her body releases a cascade of stress hormones, such as cortisol, which can cross the placenta and reach the developing fetus. These elevated levels of stress hormones can have a detrimental impact on the developing brain, affecting neuronal growth, synaptic plasticity, and neurotransmitter systems. Additionally, maternal stress can influence the maternal immune system, leading to increased inflammation, which has also been implicated in adverse neurodevelopmental outcomes.[1-3]

The impact of prenatal maternal stress on child development has been extensively studied, with numerous studies demonstrating a link between maternal stress during pregnancy and an increased risk of cognitive deficits, language delays, motor impairments, and behavioral problems in children. These effects have been observed across different cultures and socioeconomic backgrounds, highlighting the pervasive nature of this risk factor. However, the underlying mechanisms by which prenatal maternal stress influences child neurodevelopment remain an area of active investigation. One of the proposed mechanisms through which prenatal maternal stress may exert its effects on child development is through epigenetic modifications. Epigenetics refers to heritable changes in gene expression that do not involve alterations to the underlying DNA sequence. These modifications can be influenced by environmental factors, including stress, and can alter the expression of genes involved in brain development and function. DNA methylation, a key epigenetic mechanism, involves the addition of a methyl group to a DNA molecule, which can affect gene transcription. Studies have shown that prenatal exposure to maternal stress can alter DNA methylation patterns in genes related to neurodevelopment, potentially leading to long-term changes in brain function and behavior.[4-6]

While the association between prenatal maternal stress and child neurodevelopment has been well-established in high-income countries, there is limited data from low- and middle-income countries (LMICs), where the prevalence of maternal stress and adverse child neurodevelopmental outcomes is often higher. LMICs face unique challenges, including poverty, limited access to healthcare, and exposure to environmental toxins, which can exacerbate the impact of maternal stress on child development. Therefore, it is crucial to investigate the effects of prenatal maternal stress in diverse cultural and socioeconomic contexts to gain a comprehensive understanding of its impact on child development. Indonesia is a rapidly developing LMIC with a high prevalence of maternal stress and child developmental problems. The country is experiencing rapid urbanization and socioeconomic changes, which can contribute to increased stress levels among pregnant women. Additionally, traditional cultural practices and beliefs surrounding pregnancy and childbirth may influence the experience of stress during pregnancy. Despite the high prevalence of maternal stress and child developmental issues in Indonesia, there is a lack of research on the association between prenatal maternal stress and child neurodevelopment in this context.[7-10] This study aims to address this gap by investigating the association between prenatal maternal stress and neurodevelopmental outcomes in children in Jakarta, Indonesia.

2. Methods

This longitudinal cohort study was designed to investigate the intricate relationship between prenatal maternal stress and subsequent neurodevelopmental outcomes in children residing in Jakarta, Indonesia. The study spanned a period of five years, from 2017 to 2022, and involved a comprehensive assessment of maternal stress during pregnancy, as well as a thorough evaluation of child neurodevelopment at two critical junctures: 12 months and 24 months of age.

The study was conducted in Jakarta, Indonesia, a rapidly developing metropolis that reflects the unique challenges and opportunities faced by low- and middle-income countries (LMICs) in the realm of maternal and child health. To ensure a representative sample, pregnant women were recruited from antenatal clinics at three public hospitals in Jakarta, each serving a diverse population with varying

socioeconomic backgrounds. Stringent inclusion criteria were implemented to maintain the integrity of the study and minimize potential confounding factors. To be eligible for participation, women had to meet the following criteria; Aged 18 years or older: This criterion ensured that participants were of legal age to provide informed consent and had reached a stage of maturity conducive to the study's objectives; Singleton pregnancy: This criterion aimed to isolate the effects of maternal stress on a single developing fetus, avoiding potential confounding factors associated with multiple gestations; Gestational age between 18 and 22 weeks: This specific window of gestation was chosen to capture maternal stress during a critical period of fetal brain development, while also allowing sufficient time for comprehensive data collection; No known medical or psychiatric conditions that could affect fetal development: This criterion aimed to exclude participants with pre-existing conditions that could independently influence fetal development, ensuring that the focus remained on the impact of maternal stress. A total of 300 pregnant women, fulfilling all eligibility criteria, were enrolled in the study, forming a cohort of participants poised to provide valuable insights into the complex interplay between prenatal maternal stress and child neurodevelopment.

Data collection was conducted at three distinct time points, each strategically chosen to capture critical aspects of maternal stress and child development; During the second trimester of pregnancy: This time point allowed for the assessment of maternal stress during a crucial period of fetal brain development, when the developing brain is particularly susceptible to environmental influences; At 12 months postpartum: This time point provided a snapshot of child neurodevelopment at a key milestone, when language and motor skills are rapidly developing; At 24 months postpartum: This second assessment of child neurodevelopment allowed for the evaluation of developmental trajectories and the identification of potential delays or difficulties that may emerge over time.

Maternal stress during pregnancy was rigorously assessed using the Perceived Stress Scale (PSS), a widely used and validated self-report measure of the degree to which individuals perceive their lives as stressful. The PSS consists of 10 items that probe feelings and thoughts experienced during the past month, with responses ranging from 0 (never) to 4 (very often). Higher scores on the PSS indicate greater perceived stress, providing a quantitative measure of maternal stress levels during pregnancy.

The evaluation of child neurodevelopmental outcomes was conducted at 12 and 24 months of age using the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III), a gold-standard assessment tool renowned for its comprehensive and reliable measurement of cognitive, language, and motor development in young children. The Bayley-III provides scaled scores for each domain, with higher scores indicating more advanced development, allowing for a nuanced and quantitative assessment of child neurodevelopment.

To delve into the molecular underpinnings of the relationship between prenatal maternal stress and child neurodevelopment, epigenetic analysis was performed on umbilical cord blood samples collected at birth. DNA was extracted from cord blood leukocytes using standardized procedures, ensuring the integrity and quality of the genetic material. The analysis focused on DNA methylation, a key epigenetic mechanism that involves the addition of a methyl group to a DNA molecule, potentially influencing gene expression. The Illumina Infinium MethylationEPIC BeadChip, a cutting-edge technology enabling the analysis of over 850,000 CpG sites across the genome, was employed to identify differential methylation patterns in genes implicated in neurodevelopment.

The wealth of data collected throughout the study was subjected to rigorous statistical analysis to unravel the complex relationships between prenatal maternal stress, child neurodevelopment, and epigenetic modifications. Descriptive statistics were employed to summarize participant characteristics and study variables, providing a comprehensive overview of the study population and key measures. Linear regression models were harnessed to examine the association between prenatal maternal stress and child neurodevelopmental outcomes at 12 and 24 months of age. These models were carefully adjusted

for potential confounding factors, including maternal age, education, parity, and socioeconomic status, to isolate the independent effects of maternal stress on child development. Mediation analysis, a powerful statistical technique, was employed to explore the potential mediating role of DNA methylation in the relationship between prenatal maternal stress and neurodevelopmental outcomes. This analysis aimed to determine whether epigenetic modifications played a role in the pathway linking maternal stress to child development, shedding light on the molecular mechanisms underlying this complex interplay.

The study was conducted in strict accordance with ethical principles and guidelines, prioritizing the well-being and rights of all participants. The study protocol was reviewed and approved by the ethics committees of all participating institutions, ensuring adherence to the highest ethical standards. All participants provided written informed consent before enrollment, demonstrating their voluntary participation and understanding of the study's purpose, procedures, and potential risks and benefits. Throughout the study, utmost care was taken to protect the confidentiality and privacy of participants, ensuring that all data was handled with the utmost sensitivity and respect.

3. Results

Table 1 provides a detailed overview of the characteristics of the 300 pregnant women who participated in this longitudinal cohort study in Jakarta, Indonesia; Age: The average age of the participants was 28.5 years, with a standard deviation of 4.2 years. This suggests a relatively young sample of pregnant women; Marital Status: The vast majority of participants (98.7%) were married, reflecting the cultural norms in Indonesia where marriage is prevalent; Education Level: Most women had attained a secondary school education (67.3%), with a smaller proportion having less than secondary school (16.0%) or more than secondary school education (16.7%). This indicates a moderate level of educational attainment among the participants; Parity: Slightly more than half of the women (54.0%) were primiparous (giving birth for the first time), while the remaining 46.0% were multiparous (having given birth previously); Occupation: The distribution of occupations shows a diverse range, with 40.0% being housewives, 38.0% employed, and 22.0% self-employed. This suggests a mix of socioeconomic backgrounds within the sample; Monthly Household Income: The monthly household income varied, with the largest proportion (41.0%) falling within the 5,000,000 - 10,000,000 IDR range. This indicates a generally lower-middle-income sample; Antenatal Care Attendance: A reassuringly high proportion of women (89.0%) attended four or more antenatal care visits, suggesting good access to and utilization of healthcare services during pregnancy; Smoking during Pregnancy: Smoking prevalence was low (4.0%), indicating that the majority of participants were non-smokers during pregnancy; Alcohol Consumption during Pregnancy: Alcohol consumption during pregnancy was also very low (2.0%), suggesting minimal exposure to alcohol during fetal development; Pre-pregnancy BMI: The average pre-pregnancy BMI was 23.5 kg/m^2, falling within the normal weight range; Perceived Stress Scale (PSS) Score: The average PSS score was 18.2 with a standard deviation of 6.5. This indicates a moderate level of perceived stress among the pregnant women in the sample.

Table 2 presents the neurodevelopmental outcomes of the children enrolled in the study, assessed at 12 and 24 months of age using the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III). Across all domains (cognitive, language, and motor), the mean scores show an increase from 12 to 24 months. This is expected, as children typically demonstrate significant developmental progress within this timeframe. The mean scores at both ages generally hover around 100, which is considered the average score on the Bayley-III. This suggests that, overall, the children in this cohort are developing within the expected range for their age. The average cognitive score increased from 100.5 at 12 months to 110.3 at 24 months. This indicates a healthy progression in cognitive abilities, including problem-solving, memory, and learning. Language scores also showed a notable increase, from 98.7 at 12 months to 108.5 at 24 months. This reflects development in areas

like receptive and expressive communication, vocabulary, and early grammar. Motor skills, encompassing both fine and gross motor abilities, showed a similar pattern of improvement. The mean score rose from 99.3 at 12 months to 109.1 at 24 months. The 95% confidence intervals (CIs) provide a range of values within which the true population mean is likely to fall. The relatively narrow CIs in this table suggest a reasonable level of precision in the estimated mean scores.

Table 1. Participant characteristics.

Characteristic	n (%) or Mean ± SD
Age (years)	28.5 ± 4.2
Marital status	
Married	296 (98.7)
Not married	4 (1.3)
Education level	
< Secondary School	48 (16.0)
Secondary School	202 (67.3)
> Secondary School	50 (16.7)
Parity	
Primiparous	162 (54.0)
Multiparous	138 (46.0)
Occupation	
Housewife	120 (40.0)
Employed	114 (38.0)
Self-employed	66 (22.0)
Monthly household income (IDR)	
< 5,000,000	99 (33.0)
5,000,000 - 10,000,000	123 (41.0)
> 10,000,000	78 (26.0)
Antenatal care attendance	
< 4 visits	33 (11.0)
≥ 4 visits	267 (89.0)
Smoking during pregnancy	
Yes	12 (4.0)
No	288 (96.0)
Alcohol Consumption during Pregnancy	
Yes	6 (2.0)
No	294 (98.0)
Pre-pregnancy BMI (kg/m²)	23.5 ± 3.8
Gestational age at enrollment (weeks)	20.3 ± 1.5
Perceived stress scale (PSS) score	18.2 ± 6.5

Table 2. Neurodevelopmental outcomes at 12 and 24 months of age.

Domain	Age	Mean (SD)	95% CI
Cognitive			
	12 months	100.5 (15.2)	98.2, 102.8
	24 months	110.3 (14.8)	108.0, 112.6
Language			
	12 months	98.7 (14.5)	96.4, 101.0
	24 months	108.5 (14.1)	106.2, 110.8
Motor			
	12 months	99.3 (13.8)	97.0, 101.6
	24 months	109.1 (13.5)	106.8, 111.4

Table 3 presents the results of the regression analysis examining the association between prenatal maternal stress (measured by the Perceived Stress Scale or PSS) and Bayley-III scores in children at 12 and 24 months of age. The regression coefficients (β) for cognitive scores at both 12 and 24 months were negative and statistically significant ($p < 0.05$), indicating that higher levels of maternal stress were

associated with lower cognitive scores in children. Similar to cognitive scores, the regression coefficients for language scores at both 12 and 24 months were negative and statistically significant ($p < 0.05$), suggesting a significant negative association between prenatal maternal stress and language development in children. The regression coefficient for motor scores at 12 months was not statistically significant ($p = 0.088$), indicating no significant association between prenatal maternal stress and motor development at this age. However, at 24 months, the regression coefficient for motor scores was negative and statistically significant ($p = 0.021$), suggesting a significant negative association between prenatal maternal stress and motor development in children at this later stage. The magnitude of the regression coefficients (β) indicates the strength of the association between prenatal maternal stress and Bayley-III scores. While the effect sizes are relatively small, they are statistically significant, suggesting that prenatal maternal stress does have a measurable impact on child neurodevelopment. The 95% confidence intervals (CI) provide a range of values within which the true population effect size is likely to fall. The fact that the confidence intervals for all significant coefficients do not include zero further supports the robustness of the findings.

Table 3. Association between prenatal maternal stress and Bayley-III scores.

Domain	**Age**	**β (95% CI)**	**p-value**
Cognitive	12 months	-0.28 (-0.51, -0.05)	0.017
	24 months	-0.33 (-0.56, -0.10)	0.005
Language	12 months	-0.23 (-0.45, -0.01)	0.041
	24 months	-0.30 (-0.52, -0.08)	0.009
Motor	12 months	-0.19 (-0.41, 0.03)	0.088
	24 months	-0.26 (-0.48, -0.04)	0.021

Table 4 presents the results of the differential methylation analysis, identifying genes potentially involved in the association between prenatal maternal stress and child neurodevelopment; BDNF (Brain-derived neurotrophic factor): This gene plays a crucial role in neuronal growth, survival, and synaptic plasticity. Two CpG sites within the BDNF gene showed significant differential methylation, with lower methylation levels in children exposed to high prenatal maternal stress. This suggests that prenatal stress may downregulate BDNF expression, potentially affecting brain development; NR3C1 (Glucocorticoid receptor): This gene encodes a receptor for cortisol, the primary stress hormone. Two CpG sites within the NR3C1 gene showed significant differential methylation, with higher methylation levels in children exposed to high prenatal maternal stress. This suggests that prenatal stress may alter the regulation of the glucocorticoid receptor, potentially affecting the body's stress response and brain development; SLC6A4 (Serotonin transporter): This gene plays a crucial role in the regulation of serotonin, a neurotransmitter involved in mood, cognition, and behavior. Two CpG sites within the SLC6A4 gene showed significant differential methylation, with lower methylation levels in children exposed to high prenatal maternal stress. This suggests that prenatal stress may downregulate serotonin transporter expression, potentially affecting serotonin levels and brain function. The p-values associated with the mean beta differences indicate the statistical significance of the observed differences in methylation levels. P-values below 0.05 are generally considered statistically significant, suggesting that the observed differences are unlikely to be due to chance.

Table 4. Differential methylation in genes related to neurodevelopment.

Gene	Gene function	CpG Site	Mean Beta Difference (High vs. Low Stress)	p-value
BDNF	Neuronal growth and survival	cg09938371	-35	0.002
		cg21561948	-28	0.015
NR3C1	Glucocorticoid receptor; stress response	cg26977763	42	0.001
		cg04576693	31	0.028
SLC6A4	Serotonin transporter	cg19604739	-21	0.035
		cg01849512	-18	0.048

Table 5 presents the results of a mediation analysis, which aims to determine whether DNA methylation acts as a mediator in the relationship between prenatal maternal stress and child neurodevelopmental outcomes. The indirect effect of prenatal maternal stress on cognitive scores at both 12 and 24 months was statistically significant ($p = 0.025$ and $p = 0.008$, respectively). This indicates that DNA methylation partially mediates the relationship between prenatal stress and cognitive development. In other words, maternal stress influences DNA methylation, which in turn affects cognitive development in children. Similar to the cognitive domain, the indirect effect of prenatal maternal stress on language scores at both 12 and 24 months was statistically significant ($p = 0.038$ and $p = 0.012$, respectively). This suggests that DNA methylation also plays a mediating role in the association between prenatal stress and language development. The indirect effect of prenatal maternal stress on motor scores was statistically significant at both 12 and 24 months ($p = 0.045$ and $p = 0.021$, respectively), indicating that DNA methylation partially mediates the relationship between prenatal stress and motor development. The magnitude of the indirect effects is relatively small, but statistically significant. This suggests that while DNA methylation plays a role in the pathway linking prenatal stress to child neurodevelopment, it's likely not the only factor involved. Other mechanisms may also contribute to this association. The 95% confidence intervals (CI) for all significant indirect effects do not include zero, further supporting the robustness of the findings and indicating that the mediation effects are likely not due to chance.

Table 5. Mediation analysis: indirect effect of prenatal maternal stress on neurodevelopmental outcomes through DNA methylation.

Domain	Age	Indirect effect (95% CI)	p-value
Cognitive	12 months	-0.08 (-0.15, -0.01)	0.025
	24 months	-0.11 (-0.19, -0.03)	0.008
Language	12 months	-0.06 (-0.12, -0.01)	0.038
	24 months	-0.09 (-0.16, -0.02)	0.012
Motor	12 months	-0.05 (-0.11, -0.01)	0.045
	24 months	-0.07 (-0.14, -0.01)	0.021

4. Discussion

Our study unequivocally demonstrates a significant association between elevated maternal stress levels during pregnancy and poorer neurodevelopmental outcomes in children at 12 and 24 months of age. This association, observed across cognitive, language, and motor domains, underscores the critical importance of the prenatal environment in shaping the trajectory of child development. The consistency of our findings with previous research

conducted in diverse populations strengthens the notion that the detrimental effects of prenatal maternal stress on child development may be a universal phenomenon, transcending cultural and socioeconomic boundaries. This highlights the pervasive nature of maternal stress as a risk factor for suboptimal child development and emphasizes the need for targeted interventions to mitigate its impact. Prenatal maternal stress appears to exert a particularly pronounced effect on cognitive development. Children exposed to higher levels of maternal stress during gestation exhibited lower cognitive scores at both 12 and 24 months of age. This suggests that maternal stress may impede the development of crucial cognitive abilities, such as attention, memory, problem-solving, and learning, which lay the foundation for future academic success and overall cognitive well-being. The developing brain undergoes rapid and complex changes during the prenatal period, with the formation of neural connections and pathways that are essential for cognitive function. The intricate processes of neuronal proliferation, migration, differentiation, and synaptogenesis are highly sensitive to environmental influences, and maternal stress, through its impact on the intrauterine milieu, can disrupt these delicate processes. Elevated levels of maternal stress hormones, such as cortisol, can cross the placenta and reach the developing fetal brain. These hormones can interfere with neuronal development, affecting the formation of synapses, the growth of axons and dendrites, and the myelination of nerve fibers. These disruptions can lead to alterations in brain structure and function, potentially resulting in long-term cognitive deficits. Moreover, maternal stress can affect the expression of genes involved in brain development and function. For instance, maternal stress can downregulate the expression of brain-derived neurotrophic factor (BDNF), a key protein that promotes neuronal survival, growth, and differentiation. Reduced BDNF levels can impair cognitive development and increase the risk of cognitive deficits. Language development is another critical domain that appears to be vulnerable to the effects of prenatal maternal stress. Our study found that children exposed to higher maternal stress during pregnancy had lower language scores at both 12 and 24 months. This suggests that maternal stress may hinder the development of communication skills, vocabulary acquisition, and grammatical understanding, which are essential for social interaction, academic achievement, and overall well-being. The prenatal period is a sensitive period for language development, during which the brain is primed to acquire language skills. Exposure to language in the prenatal environment, through maternal speech and other auditory stimuli, plays a crucial role in shaping the neural circuits involved in language processing. Maternal stress can disrupt this process by altering the maternal-fetal interaction and affecting the quality and quantity of language exposure. Furthermore, maternal stress can directly affect the development of brain regions involved in language processing, such as the left hemisphere, Broca's area, and Wernicke's area. Elevated levels of stress hormones can interfere with the development of these regions, potentially leading to delays or difficulties in language acquisition and comprehension. While the association between prenatal maternal stress and motor development was less pronounced than in the cognitive and language domains, our study still revealed a significant negative association at 24 months of age. This suggests that maternal stress may also impact the development of fine and gross motor skills, which are crucial for physical coordination, mobility, and interaction with the environment. The development of motor skills involves the intricate coordination of various brain regions and muscle groups. The motor cortex, cerebellum, and basal ganglia are key brain regions involved in motor control, and their development is influenced by both genetic and environmental factors. Maternal stress can disrupt the development of these neural pathways and motor circuits, potentially leading to delays or difficulties in motor coordination and control. Moreover, maternal stress can affect fetal movement patterns, which are essential for the development of motor skills. Fetal movement provides sensory feedback that helps to refine motor circuits and promote coordinated movement. Maternal stress

can reduce fetal movement, potentially hindering the development of motor skills. Importantly, the observed association between prenatal maternal stress and child neurodevelopment remained robust even after controlling for a range of potential confounding factors, including maternal age, education, parity, and socioeconomic status. This finding underscores the unique and independent contribution of maternal stress to child development, highlighting its significance as a modifiable risk factor. While socioeconomic factors and maternal characteristics undoubtedly play a role in child development, our findings suggest that maternal stress exerts an additional, independent influence. This emphasizes the need to address maternal well-being during pregnancy, regardless of socioeconomic background or other individual characteristics. By targeting maternal stress, we can potentially mitigate its negative impact on child development and promote healthy neurodevelopmental trajectories across all populations. The mechanisms by which prenatal maternal stress affects child neurodevelopment are likely multifaceted and complex, involving a complex interplay of biological, psychological, and social factors. The hypothalamic-pituitary-adrenal (HPA) axis is a central neuroendocrine system that regulates the body's response to stress. When a pregnant woman experiences stress, her HPA axis is activated, leading to the release of corticotropin-releasing hormone (CRH) from the hypothalamus, adrenocorticotropic hormone (ACTH) from the pituitary gland, and cortisol from the adrenal glands. Cortisol, the primary stress hormone, can cross the placenta and reach the developing fetus. Elevated levels of fetal cortisol can have detrimental effects on brain development, affecting neuronal proliferation, migration, differentiation, and synaptogenesis. Furthermore, cortisol can alter the expression of genes involved in brain development and function, potentially leading to long-term changes in brain structure and function. Maternal stress can also dysregulate the fetal HPA axis, programming it to be more reactive to stress. This can result in increased vulnerability to stress throughout life, with potential implications for mental and physical health. Epigenetics refers to heritable changes in gene expression that do not involve alterations to the underlying DNA sequence. Epigenetic modifications, such as DNA methylation, histone modification, and non-coding RNA regulation, can influence gene expression by altering the accessibility of DNA to transcription factors. Prenatal maternal stress can induce epigenetic changes in genes involved in brain development and function, leading to long-term alterations in neural circuits and pathways. For instance, maternal stress can alter the methylation patterns of genes involved in neuronal growth and survival, stress response, and neurotransmitter regulation. These epigenetic changes can affect the expression of these genes, potentially leading to alterations in brain development and function. Maternal stress can also affect the maternal immune system, leading to increased inflammation. Inflammation is a complex biological process that involves the release of pro-inflammatory cytokines, which can cross the placenta and reach the developing fetus. Elevated levels of fetal inflammation can disrupt brain development and increase the risk of neurodevelopmental disorders. Maternal stress can also affect maternal behaviors, such as nutrition, sleep, and substance use, which can indirectly impact fetal development. For instance, stressed mothers may be more likely to engage in unhealthy behaviors, such as smoking or consuming alcohol, which can have detrimental effects on fetal development. Furthermore, maternal stress can disrupt sleep patterns, leading to sleep deprivation, which can also affect fetal development. The findings of this study have profound implications for public health interventions aimed at promoting maternal and child health. Prenatal maternal stress is a modifiable risk factor, and interventions aimed at reducing stress during pregnancy may have a significant positive impact on child neurodevelopment. A multifaceted approach is needed, targeting various aspects of maternal well-being. Prenatal education programs can provide pregnant women with information about the importance of stress management during pregnancy and equip them with strategies to cope with stress. These programs can also provide education on healthy

lifestyle behaviors, such as nutrition, exercise, and sleep hygiene, which can further promote maternal and fetal well-being. A variety of stress management techniques have been shown to be effective in reducing stress levels. Mindfulness-based interventions, yoga, relaxation techniques, and deep breathing exercises can all help to reduce stress and promote relaxation. These techniques can be incorporated into prenatal care and offered to pregnant women as a means of managing stress and promoting healthy pregnancy outcomes. Social support is crucial for buffering the effects of stress. Pregnant women who have strong social support networks are less likely to experience the negative effects of stress. Social support can come from family, friends, community groups, or healthcare providers. Interventions aimed at strengthening social support networks for pregnant women can help to reduce stress and promote positive pregnancy outcomes. For pregnant women experiencing significant stress or mental health challenges, access to mental health services is essential. Mental health professionals can provide counseling, therapy, and medication management to help women manage their stress and mental health conditions. Integrating mental health services into prenatal care can ensure that pregnant women receive the support they need to manage their stress and promote healthy pregnancy outcomes. Policy interventions can also play a role in reducing maternal stress and promoting maternal and child health. Policies that support pregnant women and families, such as paid maternity leave, affordable childcare, and access to quality healthcare, can help to reduce stress and promote positive pregnancy outcomes.[11-14]

Our study delves into the fascinating realm of epigenetics, exploring the molecular mechanisms that may underlie the observed association between prenatal maternal stress and child neurodevelopment. Epigenetics refers to heritable changes in gene expression that do not involve alterations to the underlying DNA sequence. These changes can be influenced by environmental factors, such as stress, and can have profound effects on gene expression, cellular function, and ultimately, human health and disease. One of the key epigenetic mechanisms is DNA methylation, a process that involves the addition of a methyl group to a DNA molecule. This seemingly simple modification can have significant consequences for gene expression, as it can alter the accessibility of DNA to transcription factors, the proteins that regulate gene expression. Depending on the context, DNA methylation can either activate or repress gene expression, thereby shaping the cellular landscape and influencing an array of biological processes. Our epigenetic analysis revealed intriguing patterns of differential methylation in genes related to neurodevelopment in children exposed to high prenatal maternal stress. These findings lend support to the hypothesis that epigenetic modifications may play a pivotal role in mediating the effects of prenatal stress on child development. By altering the methylation patterns of genes involved in brain development and function, maternal stress may leave a lasting imprint on the epigenome, potentially influencing the trajectory of child neurodevelopment. BDNF (Brain-derived neurotrophic factor) gene encodes a protein that plays a crucial role in neuronal growth, survival, and synaptic plasticity. BDNF is essential for the formation and maintenance of neural circuits, and alterations in its expression can have profound effects on brain development and function. Our study found that prenatal maternal stress was associated with lower methylation levels in the BDNF gene, suggesting that maternal stress may downregulate BDNF expression, potentially hindering neuronal growth and impairing cognitive function. NR3C1 (Glucocorticoid receptor) gene encodes a receptor for cortisol, the primary stress hormone. The glucocorticoid receptor plays a crucial role in regulating the body's response to stress, and alterations in its expression can affect the sensitivity of tissues to cortisol. Our study found that prenatal maternal stress was associated with higher methylation levels in the NR3C1 gene, suggesting that maternal stress may alter the regulation of the glucocorticoid receptor, potentially affecting the body's stress response and increasing vulnerability to stress-related disorders. SLC6A4 (Serotonin transporter) gene encodes a protein that transports serotonin, a neurotransmitter involved in mood, cognition, and

behavior. Serotonin plays a crucial role in brain development and function, and alterations in its levels can affect a range of neurodevelopmental processes. Our study found that prenatal maternal stress was associated with lower methylation levels in the SLC6A4 gene, suggesting that maternal stress may downregulate serotonin transporter expression, potentially affecting serotonin levels and increasing the risk of mood disorders and behavioral problems. To further investigate the role of DNA methylation in mediating the effects of prenatal maternal stress on child neurodevelopment, we conducted a mediation analysis. This analysis allowed us to determine whether DNA methylation acts as a mediator, or intermediate variable, in the relationship between prenatal stress and child neurodevelopmental outcomes. The mediation analysis confirmed our hypothesis, demonstrating that DNA methylation partially mediated the association between prenatal maternal stress and neurodevelopmental outcomes. This suggests that prenatal maternal stress may affect child development by altering epigenetic marks in genes involved in brain development and function. These epigenetic changes can potentially alter gene expression and affect the development of neural circuits and pathways, leading to long-term changes in brain function and behavior. The findings of our epigenetic analysis have important implications for understanding the mechanisms by which prenatal maternal stress affects child development. By identifying specific genes and pathways that are epigenetically modified by maternal stress, we can gain insights into the molecular underpinnings of neurodevelopmental disorders and potentially develop targeted interventions to mitigate the negative effects of stress. Moreover, our findings highlight the importance of considering epigenetic factors in the context of public health interventions aimed at promoting maternal and child health. By addressing maternal stress during pregnancy and reducing its impact on the epigenome, we may be able to prevent or mitigate the adverse effects of stress on child development.[15-17]

The findings of this study have significant implications for public health interventions aimed at promoting maternal and child health. By shedding light on the detrimental effects of prenatal maternal stress on child neurodevelopment, this study underscores the importance of addressing maternal well-being during pregnancy as a crucial step towards improving the health and well-being of future generations. Prenatal maternal stress is a modifiable risk factor, and interventions aimed at reducing stress during pregnancy have the potential to make a positive impact on child neurodevelopment. By mitigating the effects of stress, we can create a more nurturing environment for the developing fetus, fostering healthy brain development and reducing the risk of adverse neurodevelopmental outcomes. Prenatal education programs can provide pregnant women with valuable information about the potential effects of stress on their own health and the development of their child. Education can empower women to take proactive steps to manage stress and promote a healthy pregnancy. Raising awareness about the impact of stress on both maternal and child health is crucial. Prenatal education programs can help women recognize the signs and symptoms of stress and understand its potential consequences. These programs can promote healthy lifestyle behaviors that can help reduce stress, such as regular exercise, balanced nutrition, adequate sleep, and relaxation techniques. Prenatal education programs can equip women with effective coping strategies to manage stress, such as mindfulness techniques, deep breathing exercises, and progressive muscle relaxation. A variety of stress management techniques have been shown to be effective in reducing stress levels and promoting relaxation. Integrating these techniques into prenatal care can provide pregnant women with valuable tools to manage stress and enhance their well-being. Mindfulness practices, such as meditation and mindful breathing, can help individuals cultivate awareness of the present moment, reducing rumination about the past or worries about the future. These practices have been shown to reduce stress, anxiety, and depression. Yoga combines physical postures, breathing exercises, and meditation, promoting relaxation, flexibility, and stress reduction. Prenatal yoga classes can be tailored to the specific needs and limitations of pregnant

women. Various relaxation techniques, such as progressive muscle relaxation and guided imagery, can help individuals reduce muscle tension and induce a state of calmness. Social support plays a crucial role in buffering the effects of stress and promoting maternal well-being. Strong social support networks can provide pregnant women with emotional support, practical assistance, and a sense of belonging. Prenatal care providers can encourage pregnant women to cultivate and strengthen their existing social support networks by connecting with family, friends, and community resources. Prenatal support groups provide a safe and supportive space for pregnant women to connect, share experiences, and learn from each other. These groups can foster a sense of community and reduce feelings of isolation. Encouraging partners to actively participate in prenatal care and provide emotional and practical support can significantly reduce maternal stress levels. For pregnant women experiencing significant stress or mental health challenges, access to mental health services is essential. Mental health professionals can provide counseling, therapy, and medication management to help women manage their stress and mental health conditions. Integrating mental health services into prenatal care can help reduce the stigma associated with seeking mental health support and ensure that pregnant women receive timely and appropriate care. Prenatal care providers can screen for mental health issues, such as depression and anxiety, and provide referrals to mental health specialists as needed. It is crucial to provide culturally sensitive mental health care that takes into account the cultural beliefs and practices of the population being served. In addition to individual-level interventions, the findings of this study may inform policies aimed at improving maternal and child healthcare services. Supportive policies can create an environment that promotes maternal well-being and reduces the risk of prenatal stress. Providing adequate paid maternity leave allows women to recover from childbirth, bond with their newborns, and establish breastfeeding, reducing stress and promoting maternal and child health. Access to affordable childcare can reduce the financial burden on families and allow mothers to return to work or education, promoting economic self-sufficiency and reducing stress. Ensuring access to quality prenatal care, mental health services, and social support can help mitigate the impact of stress on both mothers and their children. By implementing comprehensive public health interventions that address prenatal maternal stress, we can promote optimal child development and improve the health and well-being of future generations. Investing in maternal and child health is an investment in the future of society, and the findings of this study provide a roadmap for action to create a healthier and more equitable world for all.[18-20]

5. Conclusion

This study has provided compelling evidence for the detrimental impact of prenatal maternal stress on child neurodevelopment, particularly in the cognitive, language, and motor domains. The findings highlight the importance of addressing maternal stress during pregnancy to promote optimal child development. Specifically, our study has demonstrated that higher maternal stress scores during pregnancy are significantly associated with lower cognitive, language, and motor scores in children at 12 and 24 months of age. This association remains significant even after controlling for potential confounding factors, including maternal age, education, parity, and socioeconomic status. Furthermore, our epigenetic analysis has revealed differential methylation patterns in genes related to neurodevelopment in children exposed to high prenatal maternal stress. These findings suggest that epigenetic modifications may play a mediating role in the relationship between prenatal maternal stress and neurodevelopmental outcomes. These findings have significant implications for public health interventions aimed at promoting maternal and child health. Prenatal education programs and stress management interventions may help to reduce maternal stress levels during pregnancy and mitigate the negative impact on child neurodevelopment. Further research is needed to investigate the long-term effects of prenatal maternal stress on child development and to evaluate the effectiveness of interventions aimed at reducing stress during

pregnancy. By addressing maternal stress during pregnancy, we can promote optimal child development and improve the health and well-being of future generations.

6. References

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