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MICRONUTRIENT DEFICIENCIES AND NEURODEVELOPMENTAL DELAYS IN TODDLERS: A CROSS-SECTIONAL STUDY

Original Article

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ABSTRACT

Background: Micronutrient deficiencies, particularly iron, vitamin B12, and zinc, are prevalent among children under five and are linked to impaired neurodevelopment. Despite their significance, few studies have jointly evaluated these deficiencies in relation to developmental outcomes using standardized tools.

Objective: To determine the prevalence of iron, vitamin B12, and zinc deficiencies and assess their association with neurodevelopmental delays in children aged 12–59 months.

Methods: A cross-sectional study was conducted over eight months in tertiary care hospitals in Islamabad and Rawalpindi. A total of 320 children were enrolled based on predefined inclusion criteria. Blood samples were analyzed for serum ferritin, vitamin B12, and zinc levels. Neurodevelopment was assessed using the Ages and Stages Questionnaire, Third Edition (ASQ-3), across five developmental domains. Statistical analyses included chi-square tests, t-tests, and multivariate logistic regression to identify associations between deficiencies and developmental delays, adjusting for confounders.

Results: Iron, vitamin B12, and zinc deficiencies were found in 26.3%, 20.6%, and 22.2% of participants, respectively. Developmental delays were most frequent in the fine motor (14.7%) and communication (12.8%) domains. Logistic regression analysis revealed significant associations between micronutrient deficiencies and developmental delays: iron (AOR = 2.31, 95% CI: 1.44–3.72), vitamin B12 (AOR = 1.89, 95% CI: 1.13–3.18), and zinc (AOR = 2.05, 95% CI: 1.25–3.36).

Conclusion: Micronutrient deficiencies, particularly of iron, vitamin B12, and zinc, are significantly associated with neurodevelopmental delays in young children. Routine nutritional screening and early interventions are critical to support optimal child development.

Keywords: ASQ-3, Child Development, Cross-Sectional Studies, Iron Deficiency, Micronutrients, Neurodevelopmental Disorders, Vitamin B12 Deficiency, Zinc Deficiency.

INTRODUCTION

Neurodevelopment during the first five years of life is a critical window that profoundly influences a child's cognitive, emotional, and physical growth. This period is characterized by rapid brain development, high synaptic plasticity, and the establishment of fundamental neural pathways that govern future learning and behavior. Ensuring optimal nutrition during this stage is essential, as it lays the foundation for long-term health and cognitive competence (1). Among the essential nutritional factors, micronutrients—specifically iron, vitamin B12, and zinc—play a pivotal role in supporting neurodevelopmental processes such as myelination, neurotransmitter synthesis, and synaptic connectivity. However, deficiencies in these micronutrients are alarmingly common in young children, particularly in low- and middle-income settings, and are often underdiagnosed due to the nonspecific nature of early symptoms. Iron deficiency, the most prevalent nutritional disorder worldwide, is known to impair cognitive performance, motor development, and social-emotional behaviors in infants and toddlers (2,3). Even in the absence of anemia, iron insufficiency can disrupt the function of dopamine and serotonin pathways, both of which are crucial for attention, memory, and emotional regulation. Vitamin B12, though less frequently discussed in pediatric settings, is equally critical. It is essential for the formation of myelin sheaths and the integrity of the central nervous system (4). Deficiency during early development has been associated with language delays, irritability, and even irreversible neurological damage in extreme cases. Zinc, a cofactor for numerous enzymes, contributes to neurogenesis, synaptic plasticity, and neuronal signaling. Inadequate levels have been linked to delays in motor development and attention deficits (5,6).

Existing research has highlighted the individual roles of these micronutrients in neurodevelopment, yet there remains a paucity of data exploring the co-occurrence of multiple micronutrient deficiencies and their compounded effects on developmental outcomes. Most existing studies have focused on single-nutrient interventions or clinical populations, thus failing to capture the broader, more nuanced patterns observable in general pediatric populations (7,8). Moreover, many studies in this domain lack integration with developmental assessments, limiting the translation of biochemical findings into meaningful developmental context. The relationship between micronutrient status and neurodevelopment is further complicated by socioeconomic, environmental, and genetic factors. Toddlers from underprivileged backgrounds are at heightened risk due to factors such as food insecurity, poor dietary diversity, and limited access to healthcare (9). These same children are also more vulnerable to developmental delays due to a confluence of risk factors, including maternal education, environmental stimulation, and exposure to infections. Hence, disentangling the contribution of nutritional deficiencies from other influencing variables requires a well-designed epidemiological approach (10).

A cross-sectional study design provides an appropriate framework to examine these associations in a representative population. By evaluating children across a range of nutritional and developmental statuses, it is possible to capture real-world patterns and associations that are often missed in more narrowly defined clinical trials (11,12). Furthermore, integrating micronutrient assessments with validated developmental screening tools enhances the relevance of findings for both clinical practice and public health policy. The lack of routine screening for micronutrient deficiencies and neurodevelopmental delays in many healthcare systems underscores the urgency of such research (13). Early identification and intervention are key, as delays identified during toddlerhood can often be ameliorated or even reversed with timely nutritional and developmental support. Without such data, children with potentially reversible impairments may go unnoticed until school age, when interventions are less effective and consequences more entrenched. Despite the growing acknowledgment of the importance of early childhood nutrition, there is still limited empirical evidence quantifying the joint prevalence of iron, vitamin B12, and zinc deficiencies in children under five, especially in relation to standardized measures of neurodevelopment. Addressing this knowledge gap can guide the development of comprehensive screening protocols and targeted intervention strategies, thereby promoting healthier developmental trajectories. To address this critical gap, the present cross-sectional study aims to evaluate the prevalence of iron, vitamin B12, and zinc deficiencies in children under five years of age and to investigate their association with neurodevelopmental delays. By identifying potential correlations between specific micronutrient insufficiencies and developmental outcomes, this study seeks to inform clinical guidelines and public health strategies aimed at improving child health during this foundational stage of life.

METHODS

This cross-sectional study was conducted over a period of eight months at tertiary care hospitals in Islamabad and Rawalpindi, Pakistan. The study aimed to evaluate the prevalence of iron, vitamin B12, and zinc deficiencies and their association with neurodevelopmental delays in children under five years of age. Given the objective and the expected prevalence rates of micronutrient deficiencies and developmental delays reported in similar regional studies, the required sample size was calculated using OpenEpi (version 3.01).

Assuming a 25% prevalence of iron deficiency with a 5% margin of error and 95% confidence level, the minimum required sample size was 288. Accounting for potential non-response or missing data, a final sample of 320 children was targeted for enrollment. Participants were selected through a non-probability consecutive sampling technique from pediatric outpatient and immunization clinics at the selected hospitals. Inclusion criteria were children aged 12 to 59 months who were otherwise clinically stable and accompanied by a parent or legal guardian capable of giving informed consent. Children with known chronic illnesses (such as congenital metabolic disorders, neurological syndromes, or genetic conditions), those already receiving micronutrient supplementation for the past three months, or those with acute illnesses at the time of enrollment were excluded to avoid confounding the neurodevelopmental assessment (3,14).

Following informed written consent from the guardians, participants underwent structured data collection in a dedicated consultation space within the hospital. Demographic details, dietary patterns, and perinatal histories were obtained through caregiver interviews using a pre-tested, semi-structured questionnaire administered by trained research assistants. Anthropometric measurements including weight, height, and head circumference were recorded using calibrated equipment and standardized procedures.

To assess micronutrient status, venous blood samples (3–5 mL) were drawn by certified phlebotomists using aseptic techniques. Serum ferritin, vitamin B12, and zinc levels were measured at hospital-affiliated laboratories using chemiluminescence immunoassay for ferritin and B12, and atomic absorption spectrophotometry for zinc. Reference cut-off values for deficiencies were established based on WHO guidelines and local laboratory norms: serum ferritin <12 ng/mL (without inflammation), vitamin B12 <200 pg/mL, and serum zinc <70 µg/dL. Neurodevelopmental status was evaluated using the Ages and Stages Questionnaire, Third Edition (ASQ-3), a validated developmental screening tool appropriate for children up to 66 months. The ASQ-3 covers five developmental domains: communication, gross motor, fine motor, problem solving, and personal-social skills. Caregivers completed the ASQ-3 in their native language (Urdu), with assistance provided by trained study staff to ensure comprehension and accuracy. A child was considered to have a neurodevelopmental delay if the score fell below the cut-off in any one domain as per the ASQ-3 scoring guidelines.

Data were entered and analyzed using IBM SPSS Statistics version 25. Continuous variables such as age, anthropometric indices, and micronutrient levels were described using means and standard deviations. Categorical variables including gender, feeding practices, and developmental delay status were summarized using frequencies and percentages. The normal distribution of continuous data was confirmed using the Kolmogorov-Smirnov test (14,15). To determine the prevalence of each micronutrient deficiency, point estimates with 95% confidence intervals were calculated. For assessing associations between micronutrient deficiencies and neurodevelopmental delays, chi-square tests were used for categorical comparisons. Independent sample t-tests were used to compare mean micronutrient levels between children with and without developmental delays. Binary logistic regression analysis was performed to adjust for potential confounding variables such as age, gender, socioeconomic status, and nutritional status. Adjusted odds ratios (AOR) with 95% confidence intervals were reported to quantify the strength of associations.

Ethical approval for the study was obtained from the Institutional Review Board (IRB). The study adhered strictly to the ethical standards outlined in the Declaration of Helsinki. All participants' guardians were provided with detailed information regarding the study's objectives, procedures, and potential risks, and voluntary participation was emphasized throughout. Confidentiality of participant data was maintained by assigning unique identifier codes and securing data storage on password-protected systems. Through a rigorous methodological approach encompassing objective measurement tools, validated developmental screening, and appropriate statistical analyses, this study sought to illuminate the intersection between nutritional deficiencies and developmental outcomes in a vulnerable pediatric population. The design ensures the reliability of findings and their applicability in guiding public health interventions and clinical practices.

RESULTS

The study enrolled a total of 320 children aged 12 to 59 months, with a mean age of 33.5 months. Male participants comprised 53.8% of the sample, while females made up 46.3%. The mean weight and height of the children were 12.2 kg and 87.4 cm, respectively. The majority of the children (75.6%) were classified as having normal nutritional status, whereas 24.4% were undernourished. Biochemical assessment revealed that iron deficiency was the most common micronutrient deficiency, affecting 26.3% of participants. Zinc deficiency was identified in 22.2%, while vitamin B12 deficiency was present in 20.6% of the children. These findings are detailed in Table 2. Developmental screening using the ASQ-3 tool showed that 12.8% of children exhibited delays in the communication domain, 11.3% in gross motor skills, 14.7% in fine motor skills, 11.9% in problem-solving, and 9.4% in the personal-social domain. These results are

presented in Table 3. Overall, a significant proportion of children had at least one domain with a delay. Further analysis examined the relationship between micronutrient deficiencies and neurodevelopmental outcomes. Logistic regression revealed that iron deficiency was significantly associated with developmental delays, with an adjusted odds ratio (AOR) of 2.31 (95% CI: 1.44–3.72, p = 0.001). Vitamin B12 deficiency was also significantly associated with developmental delay (AOR: 1.89, 95% CI: 1.13–3.18, p = 0.015). Similarly, zinc deficiency showed a notable association (AOR: 2.05, 95% CI: 1.25–3.36, p = 0.005), as shown in Table 4.

Table 1: Demographic Characteristics

Variable	Value
Total Participants	320
Mean Age (months)	33.5
Male (%)	172 (53.8%)
Female (%)	148 (46.3%)
Mean Weight (kg)	12.2
Mean Height (cm)	87.4
Normal Nutrition (%)	242 (75.6%)
Undernourished (%)	78 (24.4%)

Table 2: Micronutrient Deficiencies

Micronutrient	Deficient (%)	Normal (%)
Iron	84 (26.3%)	236 (73.7%)
Vitamin B12	66 (20.6%)	254 (79.4%)
Zinc	71 (22.2%)	249 (77.8%)

Table 3: Neurodevelopmental Delays by ASQ-3 Domain

Domain	Delayed (%)	Normal (%)
Communication	41 (12.8%)	279 (87.2%)
Gross Motor	36 (11.3%)	284 (88.8%)
Fine Motor	47 (14.7%)	273 (85.3%)
Problem Solving	38 (11.9%)	282 (88.1%)
Personal-Social	30 (9.4%)	290 (90.6%)

Table 4: Association Between Micronutrient Deficiencies and Developmental Delay

Micronutrient Deficiency	AOR	95% CI	p-value
Iron	2.31	1.44–3.72	0.001
Vitamin B12	1.89	1.13–3.18	0.015
Zinc	2.05	1.25–3.36	0.005

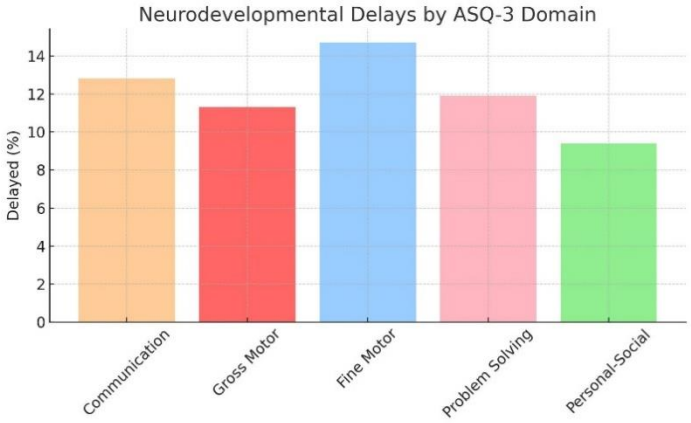

Figure 1 Neurodevelopmental Delays by ASQ-3 Domain

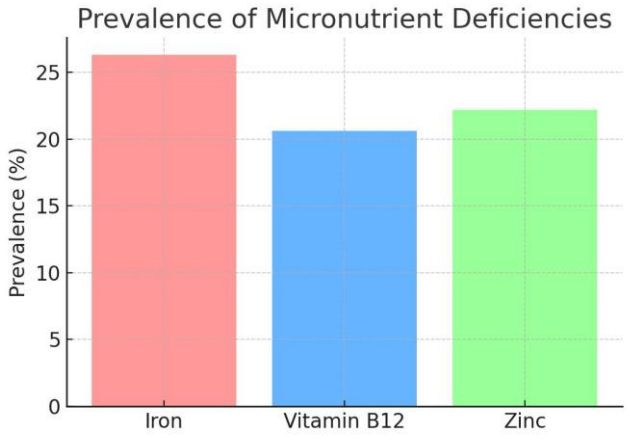

Figure 2 Prevalence of Micronutrient Deficiencies

DISCUSSION

The findings of this cross-sectional study add to the growing body of evidence linking micronutrient deficiencies with neurodevelopmental delays in early childhood. The observed prevalence of iron (26.3%), vitamin B12 (20.6%), and zinc (22.2%) deficiencies underscores the persistent burden of "hidden hunger" among young children, even within urban tertiary healthcare settings. These figures align with global estimates indicating that more than half of preschool-aged children suffer from at least one micronutrient deficiency (16). Iron deficiency emerged as the most prevalent deficiency and was significantly associated with neurodevelopmental delays (AOR: 2.31), consistent with prior studies that have shown its deleterious impact on myelination, neurotransmitter synthesis, and cognitive development (17). Similar associations were observed for vitamin B12 and zinc, both essential for neurogenesis and cellular signaling pathways critical to brain maturation. These associations mirror findings from other regional studies where hematopoietic micronutrient deficiencies significantly correlated with delayed milestones in infants (18).

The domain-specific delays noted in the ASQ-3—particularly in fine motor and communication skills—are biologically plausible, as these domains are among the most sensitive to early nutritional deficiencies. Iron, for instance, plays a known role in the dopaminergic system, which regulates motor planning and emotional expression (19). Similarly, vitamin B12 deficiency affects methylation pathways necessary for proper neurological function, and zinc is vital for synaptic plasticity and neuronal growth (20). One of the strengths of this study lies in its comprehensive assessment combining biochemical, anthropometric, and developmental domains in a single analytical framework. The use of validated tools such as ASQ-3 and standardized laboratory methods enhances the reliability of the findings. Furthermore, the adjustment for confounders like age, gender, and nutritional status in the multivariate analysis allows for more precise estimation of the independent effects of each micronutrient.

However, several limitations must be acknowledged. The cross-sectional design restricts causal inferences; although associations were strong, temporality cannot be established. There may also be residual confounding by environmental, genetic, or socio-emotional factors not measured in the study. The reliance on caregiver-reported ASQ-3 responses, despite standardized administration, introduces potential recall and reporting biases. Additionally, the sample was drawn from tertiary care hospitals, which may not fully reflect community-level prevalence or health-seeking behaviors. Despite these limitations, the study's implications are substantial. It highlights the need for routine screening of iron, B12, and zinc status in toddlers, particularly in settings where malnutrition and poor dietary diversity are prevalent. Integrating developmental surveillance with nutritional assessments can improve early identification of at-risk children and prompt timely interventions. National programs targeting child nutrition should incorporate broader coverage of micronutrient supplementation and fortification, especially for vitamin B12 and zinc, which often receive less emphasis compared to iron and vitamin A (21).

Future research should focus on longitudinal designs to determine the trajectory of neurodevelopmental outcomes in relation to micronutrient trajectories. Experimental studies exploring the effectiveness of integrated micronutrient supplementation on cognitive

and motor domains can also provide actionable insights. Moreover, context-specific data from community-based cohorts would be valuable in informing population-level interventions tailored to regional needs (22). In conclusion, the study reaffirms the pivotal role of micronutrients in early neurodevelopment and identifies iron, vitamin B12, and zinc deficiencies as significant contributors to developmental delays in young children. Bridging this nutritional gap through early detection and strategic intervention holds promise for improving child development trajectories and mitigating long-term cognitive and functional deficits.

CONCLUSION

This study established a significant association between iron, vitamin B12, and zinc deficiencies and neurodevelopmental delays in children under five. The findings emphasize the urgent need for integrated screening and nutritional interventions during early childhood. By addressing these micronutrient gaps, healthcare systems can enhance developmental outcomes and support children in reaching their full cognitive and functional potential.

AUTHOR CONTRIBUTION

Author	Contribution
Umema Mumtaz*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Durre Shehwar	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Sooraj Raja	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Nauyaan Ahmed Qureshi	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Monishka Gurdino	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Muhammad Bilal	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Muhammad Zaid Alam Siddiqui	Contributed to study concept and Data collection Has given Final Approval of the version to be published

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