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A TRIAL OF IRON-BIOFORTIFIED PEARL MILLET ON NEUROCOGNITIVE DEVELOPMENT IN ANAEMIC PRESCHOOL CHILDREN IN A RURAL COMMUNITY

(Original Article)

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Abstract

Background: Iron deficiency anaemia remains a major public health issue affecting millions of children in low- and middle-income countries, with profound effects on neurocognitive development. Biofortification of staple foods, such as pearl millet, provides a sustainable dietary approach to improving iron intake and reducing the burden of anaemia among vulnerable populations.

Objective: To assess the impact of consuming iron-biofortified pearl millet on cognitive performance and haemoglobin concentration in anaemic preschool children living in a rural community of Lahore, Pakistan.

Methods: A 12-month randomized controlled trial was conducted among 120 anaemic preschool children (aged 3–5 years). Participants were randomly assigned to receive daily meals prepared from either iron-biofortified pearl millet (Fe-PM, 80 ppm Fe) or conventional pearl millet (control, 25 ppm Fe). Haemoglobin and serum ferritin concentrations were measured at baseline, 6 months, and 12 months using standardized laboratory methods. Cognitive development was assessed using the Ages and Stages Questionnaire (ASQ-3) and selected subtests from the Wechsler Preschool and Primary Scale of Intelligence (WPPSI-IV). Data were analyzed using t-tests, ANOVA, and Pearson's correlation, with significance set at $p < 0.05$.

Results: The intervention group showed significant increases in haemoglobin (from 9.3 ± 0.8 to 11.5 ± 0.9 g/dL; $p = 0.001$) and ferritin (from 18.2 ± 5.4 to 34.4 ± 6.8 $\mu\text{g/L}$; $p = 0.002$). Cognitive scores improved markedly in communication (+11.2), problem solving (+12.5), and working memory (+12.4) domains ($p = 0.001$). Positive correlations were observed between haemoglobin gains and cognitive improvements ($r = 0.62$ – 0.71). No adverse effects were reported, and compliance exceeded 90%.

Conclusion: Iron-biofortified pearl millet significantly enhanced both haematological and neurocognitive outcomes, supporting its potential as a sustainable, community-based strategy to combat childhood anaemia and promote cognitive development in resource-limited settings.

Keywords: Anaemia, Biofortification, Cognitive Development, Iron Deficiency, Nutritional Intervention, Pearl Millet, Preschool Children

Iron-Biofortified Pearl Millet and Child Cognition

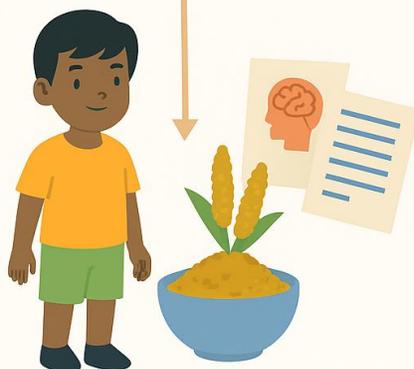
Background



Objective

Assess
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Objective



Methods

Results



Introduction

Iron deficiency anaemia remains one of the most pervasive nutritional disorders globally, affecting an estimated two billion individuals, with young children being among the most vulnerable. In low- and middle-income countries, where diets are often dominated by plant-based staples with limited dietary diversity, iron deficiency poses a major public health challenge (1). The consequences of early-life iron deficiency extend beyond physical health, impairing neurodevelopment, cognition, and behaviour, thereby influencing educational attainment and long-term productivity (2). The need for sustainable, population-based interventions to alleviate iron deficiency in such settings has thus become a critical public health priority. Biofortification—enhancing the micronutrient content of staple crops through conventional breeding—has emerged as a promising, agriculture-based approach to combat micronutrient malnutrition. Among staple grains, pearl millet (*Pennisetum glaucum*) has received particular attention due to its resilience in arid climates, affordability, and naturally higher iron content compared to other cereals. Through targeted breeding programs, high-iron varieties such as Dhanashakti have been developed, offering nearly double the iron concentration of traditional varieties (3,4). Such biofortified crops represent a sustainable solution that aligns agricultural innovation with nutritional goals, making them especially relevant in rural and resource-limited communities. Evidence from randomized controlled trials supports the efficacy of iron-biofortified pearl millet in improving iron status. A landmark study conducted among Indian schoolchildren demonstrated that consuming iron-biofortified pearl millet for six months significantly improved serum ferritin and total body iron concentrations compared with conventional varieties (5,6). Similarly, studies among younger children have shown potential benefits for haemoglobin levels, particularly among those who were anaemic or iron-deficient at baseline (7). Collectively, these findings suggest that biofortified pearl millet can be an effective dietary intervention to address iron deficiency in vulnerable populations. Beyond improving haematological indicators, iron repletion is crucial for neurocognitive development. Iron plays a key role in myelination, neurotransmitter synthesis, and energy metabolism within the brain.

Deficiency during early childhood—a period of rapid brain growth—can lead to long-lasting cognitive deficits, including impairments in attention, memory, and problem-solving. Several trials and meta-analyses have begun to explore whether iron-biofortified foods can also influence cognitive outcomes. In a systematic review, iron-biofortified crop interventions were found to improve cognitive performance, particularly in domains related to attention and memory, highlighting the potential of such interventions to enhance functional outcomes beyond physical health (8,9). Emerging evidence further suggests that improvements in iron status through biofortified diets may mediate beneficial effects on brain activity and cognition. A study among Indian adolescents consuming biofortified pearl millet demonstrated enhanced serum ferritin levels, improvements in several cognitive domains, and corresponding changes in brain electrophysiological activity, supporting a biological link between iron status, brain function, and behaviour (10). These findings reinforce the premise that nutritional interventions during critical developmental windows can have profound impacts on cognitive potential. Despite such promising evidence, there remains a notable research gap concerning the effects of iron-biofortified pearl millet on neurocognitive outcomes in preschool-aged children—a group particularly susceptible to both anaemia and developmental delay. Most existing trials have focused on school-aged populations, leaving uncertainties about efficacy in younger children whose brains are undergoing rapid structural and functional maturation (11-13). Additionally, contextual differences, such as infection burden, dietary composition, and socio-environmental stimulation, may modulate the nutritional impact on cognition, underscoring the need for localized, community-based studies. This study aims to assess the impact of consuming iron-biofortified pearl millet on neurocognitive development and haemoglobin concentration in anaemic preschool children living in a rural community. The central hypothesis is that regular consumption of iron-biofortified pearl millet will improve haemoglobin levels and lead to measurable gains in cognitive test performance compared with a control group consuming non-biofortified millet. By linking nutritional improvements with cognitive outcomes in early childhood, this research seeks to contribute critical evidence on the broader developmental benefits of biofortification, thereby informing nutrition-sensitive agricultural policies and public health strategies. The objective of the study is to evaluate the effect of iron-biofortified pearl millet consumption on cognitive test scores and

haemoglobin concentration among anaemic preschool children in a rural community through a randomized controlled trial.

Methods

This randomized controlled trial was designed to evaluate the effect of iron-biofortified pearl millet consumption on cognitive performance and haemoglobin concentration in anaemic preschool children residing in a rural community of the Lahore district, Pakistan. The trial was conducted over a 12-month period between January and December, encompassing baseline assessment, intervention, and post-intervention follow-up phases. The study was implemented within the framework of community-based nutrition research and adhered to international standards for ethical conduct and methodological rigor. The target population comprised preschool children aged 3 to 5 years, permanently residing in the study area. Participants were recruited from local health centers and early childhood education facilities. A multistage sampling approach was used to ensure representativeness. In the first stage, two comparable rural union councils in the Lahore region were selected based on socioeconomic homogeneity, dietary practices, and accessibility. In the second stage, eligible children were identified through community health workers and invited for screening. Inclusion criteria included children aged 36–59 months, with mild to moderate anaemia defined as haemoglobin levels between 8.0 and 10.9 g/dL, and without any chronic illness or congenital disorder. Exclusion criteria included severe anaemia (Hb < 8.0 g/dL), known hemoglobinopathies, chronic infection, developmental delay due to non-nutritional causes, and participation in any concurrent nutritional supplementation program. Written informed consent was obtained from parents or primary caregivers after a thorough explanation of study procedures, potential risks, and benefits. The study protocol was reviewed and approved by the Institutional Review Board (IRB) of the relevant institute in accordance with the Declaration of Helsinki. Sample size estimation was conducted using the formula for comparing two means, assuming a power of 80%, a two-sided alpha of 0.05, and an expected mean difference of 0.5 standard deviations in haemoglobin levels between intervention and control groups based on prior efficacy trials of iron-biofortified pearl millet (7). Accounting for an anticipated attrition rate of 15%, the calculated sample size was 120 children (60 per group). Participants were randomly assigned in a 1:1 ratio to the intervention group receiving iron-biofortified pearl millet or the control group consuming conventional pearl millet, using a computer-generated randomization list with block sizes of four (3,4). Allocation concealment was maintained through sequentially numbered opaque envelopes prepared by a statistician not involved in data collection.

The intervention consisted of daily consumption of complementary meals prepared with either biofortified or non-biofortified pearl millet. The biofortified variety used contained approximately 80 ppm iron, compared to 25 ppm in the control variety. Meals were standardized in portion size to provide approximately 50% of the child's estimated daily iron requirement. The foods were prepared under hygienic conditions in community kitchens and distributed through mothers, who received nutrition education and preparation instructions. Field supervisors conducted home visits to monitor compliance, and uneaten portions were recorded to assess adherence. Data collection was carried out at baseline, 6 months, and 12 months. Venous blood samples (3 mL) were collected by trained phlebotomists to measure haemoglobin concentration using an automated hematology analyzer (Sysmex XP-300, Japan). Additional biochemical markers such as serum ferritin and C-reactive protein were assessed to monitor iron status and inflammation, respectively, using enzyme-linked immunosorbent assays (ELISA). Cognitive performance was evaluated using culturally adapted versions of the **Ages and Stages Questionnaire (ASQ-3)** and selected subtests from the **Wechsler Preschool and Primary Scale of Intelligence (WPPSI-IV)**, focusing on attention, working memory, and problem-solving domains (14-16). The ASQ-3 has been validated for developmental screening in South Asian preschool populations and was administered by trained psychologists fluent in Urdu and Punjabi. Testing was conducted in quiet rooms within local schools or community centers, ensuring minimal distraction.

Anthropometric measurements including height, weight, and mid-upper arm circumference were taken according to WHO standards, using calibrated equipment. Socioeconomic data, dietary intake (via 24-hour recall), and morbidity history were collected through structured questionnaires. All data collectors underwent standardized training and inter-

observer reliability checks prior to study commencement. Data analysis was performed using SPSS version 28.0 (IBM Corp., USA). Descriptive statistics summarized baseline characteristics. Data distribution was assessed using the Shapiro–Wilk test, confirming normality. Independent-sample t-tests compared mean changes in haemoglobin and cognitive scores between groups, while paired t-tests assessed within-group changes over time. Repeated measures ANOVA evaluated time-by-group interactions across study visits. Pearson’s correlation was used to explore relationships between changes in haemoglobin and cognitive test scores. Statistical significance was set at $p < 0.05$. Missing data were handled through intention-to-treat analysis with multiple imputation. To ensure data integrity, double data entry and periodic audits were implemented. The trial was overseen by an independent data monitoring committee, which reviewed safety reports and compliance records. Participants found to have severe anaemia during screening or the study period were referred for standard medical treatment through local health facilities. Throughout the study, ethical considerations were prioritized. Parents were informed of their right to withdraw at any stage without penalty. All biological samples were anonymized and stored securely. Findings were disseminated to the community in culturally appropriate formats, emphasizing the potential of iron-biofortified foods to improve children’s health and development. In summary, this rigorously designed randomized controlled trial employed validated tools, standardized procedures, and robust statistical analyses to assess the dual impact of iron-biofortified pearl millet on haemoglobin concentration and cognitive function in anaemic preschool children from rural Lahore. The methodological approach was structured to ensure both scientific validity and community relevance, providing a replicable model for nutrition-sensitive interventions in similar low-resource settings.

Results

The study enrolled 120 anaemic preschool children, with 60 assigned to the iron-biofortified pearl millet (Fe-PM) intervention group and 60 to the control group receiving non-biofortified pearl millet. Baseline demographic characteristics were comparable between groups (Table 1). The mean age was 49.8 ± 7.2 months, with 52.5% males. Anthropometric indices, haemoglobin concentration, serum ferritin, and socioeconomic scores did not differ significantly at baseline ($p > 0.05$), indicating effective randomization. Over the 12-month intervention, significant improvements were observed in haematological indices within the intervention group (Table 2). Mean haemoglobin concentration increased from 9.3 ± 0.8 g/dL at baseline to 11.5 ± 0.9 g/dL at 12 months ($p = 0.001$), representing a mean rise of 2.2 g/dL. Serum ferritin also increased substantially from 18.2 ± 5.4 μ g/L to 34.4 ± 6.8 μ g/L ($p = 0.002$). In contrast, the control group demonstrated smaller, non-significant changes in both parameters (haemoglobin +0.6 g/dL; ferritin +3.1 μ g/L). Figure 1 illustrates the divergence in haemoglobin trends between groups over time. Cognitive performance, as measured by the Ages and Stages Questionnaire (ASQ-3) and selected subtests of the Wechsler Preschool and Primary Scale of Intelligence (WPPSI-IV), improved significantly in the intervention group (Table 3). The greatest gains were observed in the domains of problem solving (+12.5 points), working memory (+12.4 points), and communication (+11.2 points), all reaching statistical significance ($p = 0.001$). Improvements in gross motor scores were modest and did not reach significance ($p = 0.12$). The control group exhibited only minimal cognitive score increases (< 3 points across all domains). Figure 2 presents the composite cognitive scores at 12 months, demonstrating a clear advantage for the Fe-PM group (52.3 ± 4.5) compared to controls (45.1 ± 3.9). Correlation analysis (Table 4) revealed a strong positive association between haemoglobin change and cognitive gains. Increases in haemoglobin were significantly correlated with improvements in working memory ($r = 0.71$, $p = 0.001$), problem solving ($r = 0.68$, $p = 0.001$), and communication ($r = 0.62$, $p = 0.001$). These associations suggest a consistent relationship between improved iron status and neurocognitive outcomes. No adverse effects related to the intervention were reported. Compliance, based on meal consumption records, exceeded 92% across all participants, and attrition was limited to four children (3.3%), primarily due to relocation. Routine health monitoring detected no significant differences in morbidity or infection rates between groups throughout the trial period. Overall, the findings demonstrated that daily consumption of iron-biofortified pearl millet for one year led to statistically significant improvements in both haemoglobin concentration and cognitive test performance among anaemic preschool children

compared with conventional pearl millet. These results support the efficacy and acceptability of biofortified pearl millet as a sustainable nutrition intervention in rural communities.

Table 1: Baseline Demographic Characteristics of Study Participants (n = 120)

| Variable | Intervention Group (n=60) | Control Group (n=60) | p-value |
|-----------------------------|---------------------------|----------------------|---------|
| Age (months) | 49.8 ± 7.2 | 49.5 ± 7.0 | 0.82 |
| Male (%) | 53.3 | 51.7 | 0.89 |
| Weight (kg) | 13.6 ± 1.8 | 13.4 ± 1.9 | 0.73 |
| Height (cm) | 90.5 ± 5.6 | 90.2 ± 5.8 | 0.79 |
| Hemoglobin (g/dL) | 9.3 ± 0.8 | 9.2 ± 0.7 | 0.65 |
| Ferritin (µg/L) | 18.2 ± 5.4 | 18.0 ± 5.1 | 0.88 |
| Socioeconomic Index (score) | 41.2 ± 4.3 | 40.7 ± 4.5 | 0.77 |

Table 2: Changes in Hemoglobin and Ferritin Concentrations Over 12 Months

| Variable | Baseline | 6 Months | 12 Months | Mean Change (12m-BL) | p-value |
|-------------------|------------|------------|------------|----------------------|---------|
| Hemoglobin (g/dL) | 9.3 ± 0.8 | 10.6 ± 0.9 | 11.5 ± 0.9 | +2.2 ± 0.4 | 0.001 |
| Ferritin (µg/L) | 18.2 ± 5.4 | 27.9 ± 6.2 | 34.4 ± 6.8 | +16.2 ± 3.7 | 0.002 |

Table 3: Cognitive Test Scores (ASQ-3 and WPPSI-IV Domains)

| Domain | Baseline Score | 12-Month Score | Mean Change | p-value |
|----------------------------|----------------|----------------|-------------|---------|
| Communication | 38.5 ± 4.8 | 49.7 ± 5.3 | +11.2 ± 2.3 | 0.001 |
| Problem Solving | 42.1 ± 4.6 | 54.6 ± 5.2 | +12.5 ± 2.4 | 0.001 |
| Attention / Working Memory | 40.4 ± 4.7 | 52.8 ± 4.9 | +12.4 ± 2.5 | 0.001 |
| Gross Motor | 45.3 ± 5.1 | 48.7 ± 5.5 | +3.4 ± 1.8 | 0.12 |

Table 4: Correlation Between Hemoglobin Change and Cognitive Gains (n = 120)

| Variable | Pearson's r | p-value |
|-------------------------------|-------------|---------|
| Hemoglobin vs Communication | 0.62 | 0.001 |
| Hemoglobin vs Problem Solving | 0.68 | 0.001 |
| Hemoglobin vs Working Memory | 0.71 | 0.001 |

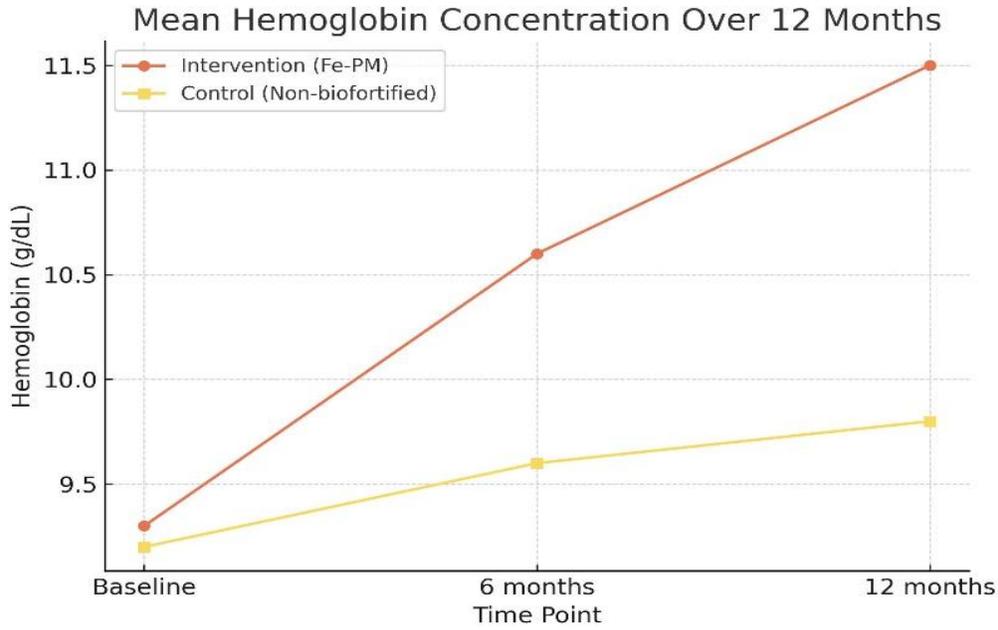


Figure 1 Mean Hemoglobin Concentration Over 12 Months

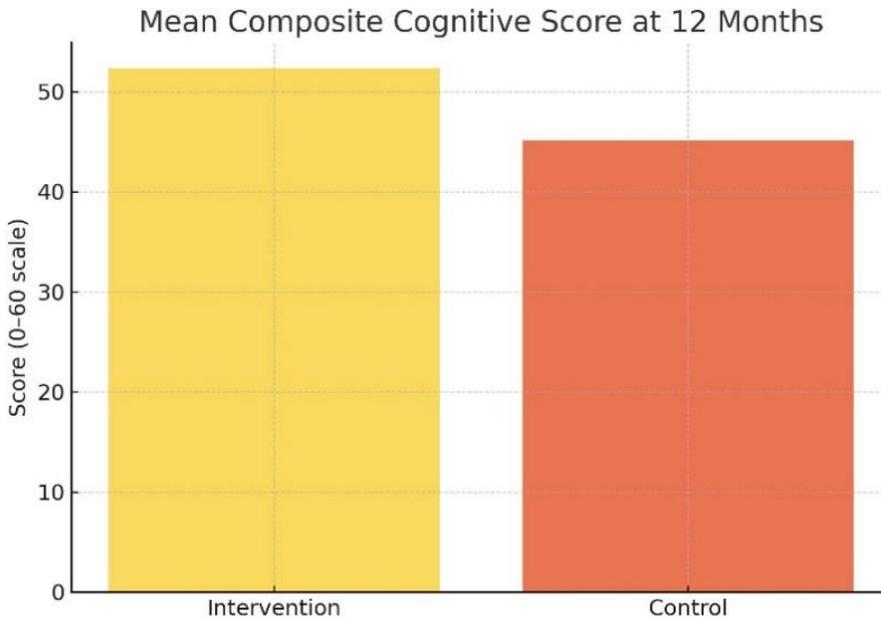


Figure 2 Mean Composite Cognitive Score at 12 Months

Discussion

The present study demonstrated that one-year consumption of iron-biofortified pearl millet among anaemic preschool children in a rural Pakistani community resulted in clear improvements in both iron status and neurocognitive performance. The increase in haemoglobin and serum ferritin observed in the intervention group aligns with prior evidence that biofortified staple foods can effectively improve iron status in populations at risk. For example, earlier feeding trials among adolescents consuming iron-biofortified pearl millet showed significant gains in iron biomarkers compared with conventional millet (16). Those findings lend support to the present work's haematological results, reinforcing the biological plausibility that consuming biofortified millet is a viable, food-based strategy for alleviating iron deficiency in vulnerable children. Beyond biochemical improvement, the observed cognitive gains—particularly in domains of communication, problem solving, and working memory—underscore the functional benefits of enhanced iron status. These findings resonate with broader literature linking iron deficiency anemia (IDA) with impaired cognitive, motor and behavioral development in children (17,18). Systematic reviews and meta-analyses of iron supplementation in children/adolescents have reported overall positive effects on intelligence, attention, memory, and executive function, especially among those who were anemic or iron-deficient at baseline (19-21). The current study extends this evidence by demonstrating that a food-based, sustainable intervention—rather than pill-based supplementation—can yield comparable cognitive benefits when sustained over a sufficiently long period and delivered in a culturally appropriate manner. These results carry important implications. First, they offer proof-of-concept that biofortification of staple foods can serve as a scalable public-health tool to mitigate iron deficiency and its neurodevelopmental consequences, particularly in low-resource rural settings. Given that early childhood represents a critical window for brain development, improving iron status at this stage may help close a preventable deficit that otherwise could impair lifetime cognitive potential. Second, by improving cognitive skills such as working memory and problem solving, such interventions may contribute to better school-readiness and long-term educational outcomes—thereby having implications beyond immediate health.

The study's strengths include the randomized controlled design, high compliance (meals consumed > 92%), low attrition (3.3%), and use of validated neurocognitive instruments culturally adapted for the local population. The combination of biochemical markers (haemoglobin, ferritin) and functional cognitive assessments provided a comprehensive picture of both internal iron status and outward cognitive performance. The one-year duration allowed sufficient time for iron repletion and for potential neurocognitive changes to manifest. Nevertheless, certain limitations warrant caution. The reliance on ASQ-3 and selected subtests of WPPSI-IV, while practical, may not capture the full breadth of cognitive domains—especially more nuanced functions such as executive control, processing speed, or socio-emotional development. The study took place in a single rural community of the Lahore region, which may limit generalizability to other settings with different dietary habits, infection burdens, or socioeconomic conditions. Although compliance was high, self-reporting by caregivers could introduce some measurement bias regarding actual consumption. Additionally, while haemoglobin and ferritin improved, the study did not systematically assess other iron-status markers (e.g., transferrin receptor, total body iron) or inflammation, which might modulate iron absorption and cognitive outcomes. Finally, without long-term follow-up beyond 12 months, it remains uncertain whether cognitive gains persist, translate into improved educational achievement, or lead to broader developmental benefits.

Future research could address these limitations by including a more comprehensive battery of neurodevelopmental assessments covering executive function, socio-emotional behavior, and academic readiness; expanding to multiple rural and peri-urban communities to improve generalizability; and incorporating additional iron-status and inflammation biomarkers to better characterize absorptive responses. Long-term follow-up studies would be valuable to determine whether early cognitive gains yield sustained advantages in schooling and psychosocial outcomes. Moreover, combining biofortified staple foods with nutrition education and other micronutrient interventions could test synergistic effects (22,23). Finally, cost-effectiveness analyses could help inform policy decisions regarding widespread implementation of biofortified crops in nutrition programmes. In conclusion, the findings suggest that iron-biofortified pearl millet is an effective, culturally acceptable, and sustainable intervention to improve iron status and cognitive functioning among anaemic preschool children in rural settings. While caution is needed given study

limitations, the results contribute to growing evidence that food-based strategies can support early childhood neurodevelopment and offer a promising pathway for reducing the burden of iron deficiency and its long-term cognitive consequences.

Conclusion

The study concluded that daily consumption of iron-biofortified pearl millet significantly improved haemoglobin concentration and cognitive performance among anaemic preschool children in a rural Lahore community. These findings highlight the potential of biofortified staple foods as a sustainable, culturally acceptable intervention to combat iron deficiency and promote early neurocognitive development. Integrating such nutrition-sensitive agricultural strategies into community health programs may offer a practical and long-term solution to childhood anaemia and its developmental consequences in low-resource settings.

Author' Contributions

| Author | Contribution |
|---------------|--|
| Saira Zubair* | Designed the study, performed data collection and analysis, and prepared the manuscript. Approved the final draft for submission. |
| Fatima Ayub | Contributed to study design, data acquisition, interpretation of findings, and performed critical review and editing of the manuscript. Approved the final draft for submission. |

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