Original Article

Impact of Zinc BioFortified Wheat Flour on the Serum Levels of Interleukin-2, Interleukin-12, and Interferon Gamma in Adolescent Girls, A Case-Control Study

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ABSTRACT

Objective: To know the Impact of Zinc BioFortified Wheat Flour on the Serum Levels of Interleukin-2, Interleukin-12, and Interferon Gamma in Adolescent Girls.

Study Design: Case-control study

Place and Duration of Study: This study was conducted at the Khyber Medical University (KMU), Peshawar from January 2023 to January 2024.

Methods: In this case-control study, adolescent girls aged 10–16 years were divided into two equal groups. The control group received regular flour, while the intervention group consumed zinc-biofortified flour. Blood samples were collected before and after a six-month period to evaluate serum levels of zinc and inflammatory markers (IL-2, IL-12, and IFN-γ), measured via ELISA.

Results: There was no statistically significant change in serum zinc or inflammatory biomarkers between groups after six months. However, a mild increase in IFN-γ levels was observed within the intervention group.

Conclusion: Zinc-biofortified wheat flour had limited impact on serum zinc and inflammatory markers over six months. Extended use may be required to yield measurable health benefits.

Key Words: Zinc biofortification, IL-2, IL-12, IFN-γ, adolescent health

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INTRODUCTION

Micronutrient deficiencies, particularly involving zinc and iron, affect over a billion people worldwide, with the highest burden in low- and middle-income populations[1]. This phenomenon, often referred to as "hidden hunger," represents a major challenge in achieving global health and nutrition targets outlined in the Sustainable Development Goals (SDGs), especially SDG 2 (Zero Hunger) and SDG 3 (Good Health and Well-being).

Zinc plays an essential role in numerous physiological functions[2]. It acts as a cofactor for over 300 enzymes and contributes to immune regulation, wound healing, oxidative stress response, and DNA repair.

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It also supports sensory functions, mental health, and reproductive outcomes. However, certain population groups such as children, the elderly, vegetarians, and individuals with low animal-source food intake remain at greater risk of zinc deficiency due to dietary limitations or increased physiological needs[3].

Chronic zinc deficiency in early life has been linked to stunted growth, weakened immunity, and neurocognitive deficits, including impaired memory and learning capacity. Moreover, zinc is crucial in modulating immune mediators like interleukin-2 (IL-2), interleukin-12 (IL-12), and interferon-gamma (IFN-γ), which are produced primarily by T lymphocytes and play key roles in cellular immune responses[4].

Several factors contribute to zinc deficiency, including poor dietary intake, impaired absorption, and the presence of dietary inhibitors such as phytates, which are common in cereal-based diets and known to hinder zinc bioavailability[5]. Addressing this public health issue requires a multifaceted approach that includes supplementation, food fortification, agricultural biofortification, and improved food processing techniques.

Among these strategies, biofortification enhancing the micronutrient content of staple crops during cultivation has emerged as a sustainable and cost-effective intervention[6]. Biofortified wheat, rice, and lentils enriched with zinc and iron have been introduced in various regions. Recent large-scale effectiveness trials are underway to evaluate the impact of these crops, particularly zinc-biofortified wheat varieties such as Zincol-2016, on improving the nutritional status of vulnerable populations, including adolescents and young children.

Zinc deficiency remains a widespread public health issue, particularly among adolescents, where rapid growth increases nutritional demands. Despite global efforts to address micronutrient malnutrition, many dietary interventions fail to achieve sustained improvements in zinc status due to poor bioavailability and dietary inhibitors such as phytates. Biofortification of staple crops offers a promising, sustainable strategy to enhance dietary zinc intake. However, the clinical effectiveness of such biofortified foods in improving immune function and inflammatory markers remains uncertain. Investigating the impact of zinc-biofortified wheat flour on immune biomarkers such as IL-2, IL-12, and IFN-γ can help determine whether this approach has measurable physiological benefits in adolescents.

METHODS

This case control study was approved by the Advanced Study and Research Board (AS &RB), Khyber Medical University (KMU) (study approval: DIR/KMU-AS&RB/CL/001843). The study was conducted at the Khyber Medical University (KMU), Peshawar from Jan 2023 to Jan 2024.

A total of 72 adolescent girls were recruited via convenience sampling. Participants were allocated into two equal groups. One group received biofortified wheat flour, while the other was provided regular flour for six months.

The levels of zinc and inflammatory biomarkers (IL-2, IL-12, and INF-γ) were assessed in both groups before and after six months of intervention.

The blood samples (2ml) were taken at the baseline and endpoint of the study to monitor zinc concentration and inflammatory biomarkers. Whole blood was extracted from the antecubital vein using a butterfly needle and plastic vacutainers (BD Diagnostics, Switzerland). Plasma and serum were separated within one hour of blood sample collection by 10 mins centrifugation at 1000 -2000 rpm, and the processed samples were stored at -80 degrees for further analysis[4].

Serum levels of zinc, IL-2, IL-12, and IFN-γ were assessed at baseline and post-intervention. Blood was drawn, processed, and stored under standardized conditions. ELISA was used to determine biomarker concentrations.

Data were analyzed using SPSS v28. Normality was tested using the Shapiro-Wilk test. Paired and independent sample t-tests were used to compare pre- and post-intervention levels.

RESULTS

A total of 72 adolescent girls aged 10 to 16 years were enrolled in this case-control study, with 36 participants in each group. The primary aim was to assess the effect of zinc-biofortified flour on serum zinc levels and selected inflammatory biomarkers (IL-2, IL-12, and IFN-γ) over a six-month period.

In the control group, mean serum zinc levels showed a slight but statistically insignificant increase from 654.67±79.19 µg/L at baseline to 674.11±154.92 µg/L after six months (p = 0.454). A significant rise was observed in IFN-γ levels, increasing from 71.75±42.91 pg/mL to 85.66±22.05 pg/mL (p = 0.049). IL-2 levels rose modestly from 380.67±125.50 pg/mL to 429.78±142.05 pg/mL, though this difference was not statistically significant (p = 0.128). IL-12 levels slightly declined from 14.47±4.91 pg/mL to 13.09±1.98 pg/mL (p = 0.123), which was also not significant.

In the intervention group, a significant increase in zinc concentration was noted, rising from 645.23±118.03 µg/L at baseline to 690.08±140.28 µg/L after six months (p < 0.001). Similarly, IFN-γ levels significantly increased from 62.46±20.87 pg/mL to 80.60±11.62 pg/mL (p < 0.001). IL-2 levels also showed a significant elevation from 345.98±77.02 pg/mL to 422.76±127.69 pg/mL (p = 0.007). However, the change in IL-12 levels from 12.80±3.38 pg/mL to 13.08±1.70 pg/mL was not statistically significant (p = 0.601).

Despite the improvements observed within the intervention group, between-group comparisons revealed no statistically significant differences after six months. Zinc levels differed by an average of 44.85±69.30 µg/L in the intervention group versus 19.44±151.75 µg/L in the control group (p = 0.365). Similarly, differences in IFN-γ (p = 0.596), IL-2 (p = 0.504), and IL-12 (p = 0.110) were not statistically significant between the two groups.

Table No.1: Intra-group Comparison of Zinc and Biomarker Levels

Parameter	Control Group (n=36)	p-value	Intervention Group (n=36)	p-value
Zinc (µg/L)	654.67±79.19 → 674.11±154.92	0.454	645.23±118.03 → 690.08±140.28	0.000
IFN-γ (pg/mL)	71.75±42.91 → 85.66±22.05	0.049	62.46±20.87 → 80.60±11.62	0.000
IL-2 (pg/mL)	380.67±125.50 → 429.78±142.05	0.128	345.98±77.02 → 422.76±127.69	0.007
IL-12 (pg/mL)	14.47±4.91 → 13.09±1.98	0.123	12.80±3.38 → 13.08±1.70	0.601

Table No.2: Post-Intervention Differences Between Groups

Variable	Control Group (Δ Mean ± SD)	Intervention Group (Δ Mean ± SD)	p-value
Zinc (µg/L)	19.44±151.75	44.85±69.30	0.365
IFN-γ (pg/mL)	13.91±40.84	18.15±24.54	0.596
IL-2 (pg/mL)	49.10±189.07	76.78±159.66	0.504
IL-12 (pg/mL)	1.38±5.26	0.29±3.28	0.110

DISCUSSION

This case-control study aimed to explore whether the consumption of zinc-biofortified wheat flour could significantly improve serum zinc levels and modulate inflammatory biomarkers, namely IL-2, IL-12, and IFN-γ, in adolescent girls over a six-month period. Although within-group analysis in the intervention group revealed statistically significant increases in zinc, IL-2, and IFN-γ, the differences between intervention and control groups were not statistically significant post-intervention.

These findings align with previous work by Escobedo-Monge et al., who reported no meaningful elevation in plasma zinc after a year-long zinc supplementation trial in children, suggesting that serum zinc levels may not always reflect dietary intake due to complex absorption dynamics or regulatory mechanisms at the cellular level[7]. Similarly, an eight-week intervention using zinc-enriched wheat flour in a randomized setting also yielded no significant changes in systemic zinc concentration, supporting the current observation that short- to mid-term dietary interventions may have limited measurable impact on serum zinc[8].

The observed rise in IFN-γ within the intervention group corresponds with experimental findings that link zinc intake to enhanced immune signaling. Zinc is known to support T-cell activity and the production of Th1-type cytokines, including IFN-γ, a key mediator in cell-mediated immunity[9]. However, as between-group comparisons failed to reach statistical significance, the modest increases may reflect individual variation, low baseline inflammation, or confounding dietary inhibitors such as phytates, which impair zinc absorption[10].

Regarding IL-2 and IL-12, the results were mixed. IL-2 showed a significant rise in the intervention group, while IL-12 remained relatively unchanged in both groups. These results are consistent with findings from studies by Raqib et al. and Ahmad et al., who also noted that zinc supplementation did not consistently enhance all cytokine levels, particularly in short-term trials[11,12]. This discrepancy might reflect the differential regulation and turnover of cytokines or the limited systemic inflammation present in the target population[13,14].

Importantly, the lack of significant difference between groups despite improvements within the intervention arm raises questions about the sensitivity of serum biomarkers in capturing localized or subclinical changes in immune function. It also suggests that a longer duration or higher dosage may be needed to elicit robust systemic effects. Moreover, factors such as dietary composition, gut health, and genetic variation in micronutrient metabolism may have influenced the outcomes.

This study contributes to a growing body of evidence suggesting that while biofortification is a promising strategy, its impact may depend on baseline nutritional status, intervention duration, and the presence of coexisting nutritional or environmental challenges. Given the mild increases observed in some immune parameters, further research using a longer follow-up period and controlling for dietary inhibitors is warranted.

CONCLUSION

This case-control study found that the six-month consumption of zinc-biofortified wheat flour resulted in modest but statistically significant increases in serum zinc, IL-2, and IFN-γ levels within the intervention group. However, when compared to the control group, these changes did not reach statistical significance. IL-12 levels remained largely unaffected throughout the study period.

These findings suggest that while zinc biofortification may have some potential to enhance immune-related biomarkers, its short-term impact on systemic zinc status and inflammatory cytokines appears limited. The absence of significant between-group differences highlights the need for longer-term interventions, higher zinc concentrations, or strategies to overcome dietary absorption barriers. Future studies should explore these possibilities to better understand the full potential of zinc biofortification in improving adolescent nutritional and immune health.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Sara Yar Khan, Sadia Fatima, Rubina Nazli
Drafting or Revising Critically:	Omer Malik, Sikandar Ali
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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