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Effect of Bio-fortified Zinc Wheat Flour (Zincol-2016) on the Serum Level of the Thyroid Hormones among Adolescent Girls

Maazullah,[1] Sadia Fatima,[2] Rubina Nazli,[2] Nicola Lowe,[3] Omar Malik,[2] Sajid Khan[1]

1. Jinnah Medical College, Peshawar, Pakistan
2. Khyber Medical University, Peshawar, Pakistan
3. University of Central Lancashire, United Kingdom

ABSTRACT

Background: Hidden hunger is an alarming concern, which has affected more than 2 billion people across the globe. Of all micronutrients, zinc is necessary for different body functions. The prevalence of zinc deficiency is high in Pakistan. Different strategies to eliminate the zinc deficiency are in practice but bio-fortification is best among all. Bio-fortified zinc flour was used for the first time in Pakistan to evaluate the effect of zinc on the serum thyroid hormones level among adolescent girls.
Objective: To measure the level of thyroid hormones after the intake of biofortified zinc wheat flour (Zincol) for 6 months by the adolescent girls of low resource community setting of newly merged districts of KPK, namely Baghbanan & Shamshato.
Materials and Methods: It was a placebo control, clustered randomized controlled trial, conducted in Baghbanan (Peshawar), Shamshato (Peshawar) and Khyber Medical University, Peshawar. The study was commenced in July, 2022 and was carried till 29th December, 2023. Adolescent girls aged 10-16 years from Peshawar were divided into 2 groups, one group was receiving Galaxy variety wheat flour & the other group was receiving bio-fortified zinc wheat flour. Blood samples were collected at baseline and post intervention point (after provision of wheat flours for six months) and these samples were analyzed through ELISA procedure, to evaluate the change in the serum thyroid profile of the participants. 60 samples were used for the study. Statistical analysis was done using SPSS 22.
Results: In this research study, after the provision of Galaxy and Zincol variety wheat flour for six months, no significant change was observed in plasma zinc concentration of the two groups. Moreover, serum 'T3' and 'T4' levels were not significantly changed whereas, only serum 'TSH' levels was significantly increased ($p<0.05$) in Zincol group.
Conclusion: By comparative analysis between Galaxy and Zincol groups, serum 'TSH' levels were observed as significantly changed whereas, plasma zinc concentration, serum 'T3' and serum 'T4' values showed no significant changes.
Keywords: Adolescent girl, Biofortification, Bizifed-2, Elisa, Galaxy, T3, T4, TSH, Zincol.

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INTRODUCTION

"Malnutrition" has been defined to indicate a shortfall, excess, or imbalance of nutrients, which has a detrimental effect on composition, function and clinical outcome of the body. Nevertheless, a growing number of people are exposed to various types of malnutrition throughout their lives and directly experience the malnutrition's double burden (DBM) due to rapid global nutrition shift.[1] When consumption and absorption of vitamins and minerals (such zinc, iodine, and iron) are insufficient to support optimum health and development, it is known as hidden hunger.[2] More than 2 billion people experience hidden hunger, especially in low- and middle-income nations.[3] Out of all the necessary micronutrients, zinc deficiency is widespread but more common in underdeveloped nations.[4] Zinc is crucial for the metabolism of proteins, fats, nucleic acids and genes.[5] Zinc deficiency has a major role in growth issues, impotence, inflammation, GIT problems or cutaneous involvement inside the human body, apoptosis, differentiation and cellular proliferation. Also, immunity, intermediate metabolism, DNA metabolism and repair, taste and cognition and behavior are a few examples of processes that call on zinc.

Correspondence: Dr. Maazullah
Jinnah Medical College, Peshawar, Pakistan
Email: drmaaz12430@gmail.com

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2.7 billion individuals worldwide lack enough zinc and it is the sixth most common factor in sickness and mortality in underdeveloped nations. Over 50% of all people on earth are at risk for low zinc intake, with the percentage of the population in each country varying from 1% to 13% in Europe and North America, 68% to 95% in South and Southeast Asia, Africa and the Eastern Mediterranean. The incidence of stunting in children and the availability of zinc in national food supply were used as two proxies, to estimate the prevalence of zinc insufficiency regionally and worldwide. Pakistan is a developing nation and numerous demographic groups suffer from nutritional deficiencies especially zinc. The National Nutrition Survey (NNS-2004) indicated that 37% of children aged 0 to 5 were zinc deficient and 45% of pregnant women lacked enough zinc. The values in the 2011 NNS survey were 41.9% and 36.5% respectively and according to Pakistan's most recent National Nutrition Survey (NNS-2018), 18.6% of children below age 5 years and 22.1% deficiency of zinc in the reproductive age women, thus even while this is an improvement, the headline of the national poll does not accurately represent the situation in some of the most disadvantaged groups.[6] Stunting (an indicator of zinc deficiency) among children under 5 years age is as high as 40% in KPK and 48.30% in the newly merged districts of KPK.[7]

The strategies recommended to combat zinc insufficiency include supplementation, fortification, diet diversification and the newly developing field of bio-fortification. Bio-fortification entails using either one or a combination of the 3 methods, which are (i) agriculture based, (ii) agronomic based and (iii) transgenic based (genetic modification) methods to increase the nutrient levels in edible plants while they are growing. It is ideal to target staple foods for bio-fortification to improve the quality of diet in poor nations because it is sustainable, very affordable and doesn't call for any modifications to the customs around food preparation and consumption. Bio-fortified zinc wheat, rice and lentils have all been introduced in China, Bangladesh, India, Pakistan and Bolivia, who also assessed the availability of iron-zinc supplemented rice in China.[8] Bio-fortified zinc wheat flour (Zincol-2016) was grown for the first time in Pakistan. The farmers were provided with zinc fertilizers and zinc foliar spray, in order to enhance the zinc content of the crop.

The thyroid is a small size and is butterfly-shaped gland, which produces and secretes thyroid hormones. The thyroid hormone regulates how quickly your body's metabolism will work. An intricate feedback loop within the human body regulates the levels of the thyroid hormones. The hypothalamus in the brain releases thyroid-releasing hormone (TRH) to begin the feedback loop, which directs the pituitary gland to generate and release thyroid-stimulating hormone (TSH). The thyroid gland is stimulated by TSH to synthesize the thyroid hormones. Thyroid hormones support healthy brain growth and performance, promotes bone health and muscle health. It controls how quickly the body's metabolism occurs. Thyroid hormones controls how proteins, fats and carbohydrates are broken down. The synthesis as well as metabolism of thyroid hormones depend on a number of trace elements. In order to maintain proper thyroid function, avoid thyroid disorders and regulate biological processes, trace minerals like zinc (Zn) and copper (Cu) are crucial. The enzyme that converts T4 (inactive form) to T3 (active form) requires zinc as a cofactor. Additionally, TRH and zinc are both involved in the transformation of pre thyrotropin releasing hormone.[8] The information transfer mechanism from tissue-specific DNA to RNA in thyroid hormone is regulated by the thyroid proteins thyroglobulin (Tg) and thyroid peroxidase (TPO). The enzyme thyroid peroxidase catalyzes the conversion of iodine to thyroglobulin (Tg), which is the precursor to T3 and T4. The deiodinase enzymes (D1D2D3), which are responsible for converting T4 to T3. The cofactor zinc has a role in the deiodinase enzyme's function.[9] In Pakistan, zinc deficiency is the emerging health issue. To overcome this, Biofortification of wheat flour with zinc was done for the first time in Pakistan, in order to evaluate the effect on the serum level of the thyroid hormones in adolescent girls.

MATERIALS AND METHODS

The study was conducted as experimental study and convenience sampling was done from adolescent girls of Baghbanan and Shamshato (Peshawar), fed with Galaxy and Zincol flour for 06 months each. The study was commenced on 6th July, 2022 at ended 29th December, 2023. GSC was approved by Graduate Study Committee (GSC) in August, 2022. Ethical Board under: KMU/IBMS/IRBE/4th meeting/2023/9821-25 approved the study in December, 2022 and the Advanced Study and Research Board under: DIR/KMU-AS&RB/EB/001951 in January, 2023. Sample selection and Elisa analysis were performed from February, 2023 till June, 2023. Thereafter, thesis write up was done from July, 2023 till December, 2023.

Adolescent girls aged 10-16 years were recruited to participate in the study sample according to the inclusion and exclusion criteria. Adolescent girls that were unmarried, non-pregnant, non-lactating and resident of Peshawar were included in the study. Girls other than specified age group that was 10-16 years, girls outside the specified area of target community and girls that are married and migrated to areas other than Peshawar, medical conditions like pregnancy and breastfeeding and adolescent girls taking medicines that have reactions with zinc or affecting the absorption of zinc were excluded from the study. There were two groups of research study, one group received bio-fortified zinc wheat flour for six months and that group was named Zincol group. The other group received non-biofortified zinc wheat flour for six months duration and named as Galaxy group. The sample size for the study was calculated with the help of Open Epi® (software for epidemiologic statistics) from a study performed about zinc, vitamin D and TSH in vertigo patients using mean ± SD value of TSH 3.95 ± 0.47 in diseased group and 3.28 ± 0.47 in placebo patients, keeping level of significance at 5% and 95% power of test.[10] The sample size for our study was 60 (36 participants included in Galaxy flour group and 24 participants included in Zincol flour group). Targeted population were selected and venous blood were collected before and after the perception of Galaxy flour and Zincol flour for six months. Whole blood was kept in Eppendorf tubes and serum plasma was separated via centrifugation and then, the processed samples were stored at -80c for further analysis. ELISA analysis was performed for investigating the serum levels of thyroid hormones in both groups (Galaxy & Zincol).

Plasma zinc concentration was measured via ICP-MS method. Whole blood (non-fasting) was drawn from the antecubital vein through a butterfly needle. Serum samples, which were centrifuged at 1000-2000 rpm for 10 minutes and frozen at -80 °C were analyzed through ELISA [DIASens T3 (DS177702), Abia T4 (DK.035.01.9) & Abia TSH (DK.006.01.3)] in June, 2023 at KMU laboratory. Serum triiodothyronine, thyroxine and TSH values were analysed from the

preserved samples through Elisa technique [DIASens T3 (DS177702), Abia T4 (DK.035.01.9) & Abia TSH (DK.006.01.3)] at KMU laboratory. Data analysis was done using SPSS version 22. For parametric test, T test was used in case of normal distribution and the normality of the data assessed using Shapiro-Wilk test. Numerical data calculated as mean and standard deviation or median for continuous variables like age, plasma zinc levels, serum levels of T3, T4 and TSH. Pre and post T3, T4 and TSH levels were analyzed through paired sample t test whereas; independent T test was performed for comparison of T3, T4 and TSH between 2 groups. Frequencies and percentages were computed from qualitative variables like gender and socio-demographic data by chi-square test. The level of significance was p=0.05.

RESULTS

The study was conducted in two rural regions of Peshawar namely; Baghbanan (5 km²) and Shamshato (4.5 km²), which are situated 3040 km southeast of Peshawar. Adolescent girls, who were un-married, non-pregnant and non-lactating, were part of the study. Adolescent girls aged between 10 to 16 years, with mean ± SD of 13.01 ± 1.21 were part of the study. Sample size for the study was kept to n = 60 (36 participants from Galaxy group and 24 participants from Zincol group).

The comparative analysis of the participants fed with 'Galaxy' wheat flour and 'Zincol' wheat flour, a significant increase was observed in mid Upper Arm Circumference [(0.54 ± 0.744 at baseline and 1.40 ± 0.890 at post intervention); (p<0.001)]. However, weight, height, BMI, waist circumference, hip circumference as well as waist to hip ratio showed no significant change while comparing the anthropometric analysis of Galaxy group and Zincol group as shown in the table 01.

Table 01. Comparative Analysis of anthropometric variables in study population (n-60)

Variable	Mean ± SD difference (Galaxy)	Mean ± SD difference (Zincol)	Significance (2-tailed)
Weight (Kg)	3.58 ± 2.26 (p<0.001)	2.96 ± 1.14 (p<0.001)	0.037
Height (cm)	0.97 ± 0.712 (p<0.001)	1.11 ± 1.04 (p<0.001)	0.060
BMI	1.25 ± 0.94 (p<0.001)	1.15 ± 0.744 (p<0.001)	0.55
MUAC (cm)	0.54 ± 0.744 (p<0.001)	1.4 ± 0.890 (p<0.001)	p<0.001
WC (cm)	2.25 ± 2.28 (p<0.001)	1.79 ± 2.28 (p<0.001)	0.777
HiC (cm)	2.25 ± 1.53 (p<0.001)	2.16 ± 1.74 (p<0.001)	0.615
WHR	0.004 ± 0.02 (p=0.242)	0.001 ± 0.021 (P=0.240)	0.513

SD= Standard deviation.

The plasma zinc concentrations, serum 'T3' levels and serum 'T4' levels showed no significant changes. However, a significant change was observed in serum 'TSH' levels [(-0.69±1.43 mIU/L in Galaxy group and 0.28±1.32 mIU/L in Zincol group); (p<0.05)], while comparing the sample analysis of Galaxy group and Zincol group as shown in the table 02.

Table 02. Comparative Analysis of research variables in study population (n=60)

Variable	Mean ± SD difference (Galaxy Group)	Mean ± SD difference (Zincol Group)	P-value
Zn (µg/L)	(621.35±114.39) - (611.40±77.53) = 9.96±81.96 (p=0.471)	(675.11±57.88) - (661.32±96.79) = 13.79±86.40 (p=0.442)	0.433
T3 (nmol/L)	(1.97±0.48) - (1.72±0.46) = 0.25±0.38 (p<0.001)	(1.93±0.446) - (1.88±0.562) = 0.052±0.42 (p=0.547)	0.782
T4 (nmol/L)	(125.49±12.84) - (121.06±12.60) = 4.43±19.04 (p=0.171)	(123.48±16.84) - (119.03±14.65) = 4.45±18.27 (p=0.245)	0.564
TSH (mIU/L)	(2.41±0.936) - (3.09±1.36) = -0.69±1.43 (p=0.007)	(2.78±1.57) - (2.50±0.733) = 0.28±1.32 (P=0.308)	0.009

SD= Standard deviation.

DISCUSSION

In this experimental study, non-significant change observed in serum zinc levels after the provision of Galaxy variety wheat flour and Zincol variety wheat flour to the participants for six months. Signorell *et al* performed a study on 273 Indian children of age group 4-12 years, to evaluate the effect of bio-fortified wheat flour on Zinc status and observed no significant change in plasma zinc concentration, which is similar to our study's finding.[11] Similarly, Gupta *et al* performed a study on adolescent girls and observed no significant change in plasma zinc concentration of the adolescent girls with the use of bio-fortified wheat flour.[12] However, Sazawal *et al* performed a study and observed that high zinc bio-fortified wheat and low zinc bio-fortified wheat both can improve plasma zinc levels significantly ($p<0.01$).[13] Likewise, Kawade *et al* performed review of studies in India, in which she did analysis on girls aged 10-16 years from western Indian schools and it was observed that zinc supplementation can improve plasma zinc level, cognitive performance and taste acuity in adolescent girls.[14]

In our randomized controlled trial, after the provision of Galaxy variety wheat flour and bio-fortified wheat flour for six months, a comparison is made between the changes observed in the two research groups. A significant change was observed in serum TSH levels however, non-significant changes were observed in the serum T3 and T4 levels, when serum ELISA analysis of Galaxy group participants were compared with that of Zincol group participants. Ahmed *et al* performed a study on 176 individuals of age group 20-55 years and observed a significant decrease ($p<0.001$) in T3, T4 and 'Zinc' levels and a highly significant increase ($p<0.0001$) in TSH levels of obese and overweight individuals as compared to the normal weight individuals.[15] Moreover, Khandhro *et al* performed a study on 60 goitrous male and 72 goitrous female patients and observed a significant increase ($p<0.01$) in TSH level in both categories, however, mean values of FT3 and FT4 were found to be lowered with the action of zinc on it.[16] Likewise, Cinar *et al* has studied 40 male individuals for determination of the effect of zinc supplementation and weight training on the thyroid metabolism of the sedentary and athletes, which concluded a significant change ($p<0.01$) in the thyroid hormone levels and forms an element supplement that can affect positively the athletic performance.[17] However, Neto *et al* carried out research on 5 healthy men, to investigate whether zinc affects TRH-stimulated TSH at different time points, when elemental zinc is administered orally and TRH administered intravenously for one minute, that resulted in non-significant correlation.[18] Likewise, Marreiro *et al* performed a study on 16 adolescents with down's syndrome, in which the effect of 30 mg zinc supplement (daily basis for 4 weeks) on thyroid hormones metabolism was to find out and it was observed that zinc was effective for zinc concentration stabilization but it did not influence thyroid hormones metabolism.[19] The studies presented for knowing any association of serum or plasma zinc to the thyroid hormones functioning and it is obvious to some extent that zinc is an important micronutrient, which can correct the disorders of thyroid gland, if it is given in proper amount for proper time and all other factors are not allowed for participation that have negative impact on zinc or thyroid hormones.

CONCLUSION

- Serum zinc levels showed no significant change in samples taken from participants fed with both varieties of flours (Non bio-fortified flour & zinc bio-fortified flour).
- A significant increase in serum T3 levels and a significant decrease in serum TSH levels were observed in samples of participants from Galaxy group.
- However, there was no significant change observed in pre and post values of serum T4 in samples from Galaxy group. Additionally, Zincol group samples showed no significant change in the serum T3, T4 and TSH levels.

Limitations

There are several limitations to our research study, which are;

- As milling process results in yield of approximately 80%, in which 20% of bran is removed and analysis of this revealed that about 50% reduction in zinc concentration occurs, when flour is obtained after the bran is removed.
- Phytate acts as inhibitor for zinc absorption and phytate is present in some seeds, nuts, legumes and unprocessed whole grains. The flour of our study was not subjected to any process through which the phytate content should be reduced. Veggies such as cauliflower, broccoli etc and fruits such as banana, apples etc and nuts like cashews and almonds are suggested to be taken by the study subjects, in order to reduce the adverse effects of phytic acid.

Recommendation

- Our study was a pilot study, which cannot be applicable to the whole population of Pakistan. For that reason, larger scale study should be performed, which can give us full assessment of plasma zinc status of Pakistan.

Ethical Consideration

This study was approved by Advanced Study and Research Board, KMU (DIR/KMU-AS&RB/EB/001951) Peshawar, Pakistan.

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Conflict of Interest

Authors declared no conflict of interest.

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DATA SHARING STATEMENT: The data that support the findings of this study are available on request from the corresponding author. The data is not publicly available due to privacy or ethical restrictions.

AUTHORS' CONTRIBUTION

The following authors fulfill authorship criteria as per ICMJE guidelines;

Maazullah, Fatima S: Substantial contribution to conception and design, drafting the manuscript, final approval, agreed to be accountable for all the work.

Nazli R, Lowe N: Substantial contribution to conception and design, data acquisition, drafting the manuscript, final approval, agreed to be accountable for all the work.

Malik O: Analysis and interpretation of data, drafting the manuscript, final approval, agreed to be accountable for all the work.

Khan S: Analysis and interpretation of data, critical revision, final approval, agreed to be accountable for all the work.