Supplementation with Iron and Zinc Selectively Improves Cognitive and Behavioral Functions in Female Adolescents

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***Abstract*—This study was designed to test the efficacy of iron, zinc and combined iron and zinc supplementation on cognitive functions and behavioral outcomes of female adolescents. A randomized, double-blind, placebo-controlled, intervention trial was conducted in Chennai, India among 4 groups of female adolescents who were aged 17 to 19 years. They were randomly assigned to any one of the four intervention groups and were given iron alone (Fe, 60 mg elemental iron), zinc alone (Zn, 30 mg elemental zinc), both iron and zinc (FeZn, 60 mg elemental iron and 30 mg elemental zinc) or placebo (PC) daily for 4 months. Hemoglobin, serum ferritin, serum zinc , 7 cognitive tests of mental speed, sustained attention, abstract reasoning, verbal and visual memory and verbal and visual recognition and three behavioral outcomes including anxiety, depression and perceived general fatigue were assessed at baseline and after 4 months of supplementation. Post intervention, the groups supplemented with Fe and FeZn scored higher on the cognitive function of visual memory than the Zn alone and PC groups. Iron and Zinc either as single supplements or in the combined form were equally efficacious in improving the cognitive function of mental speed on accuracy levels. The Fe, Zn , and FeZn groups did not improve in other cognitive measures: sustained attention, abstract reasoning immediate and delayed recall of verbal material and verbal and visual recognition compared with the PC group. Behavioral measures (state anxiety, trait anxiety and perceived general fatigue) did not differ among the groups after 4 months of supplementation. Supplementation with FeZn significantly reduced Beck Depression Inventory scores , but there was no change in the Fe, Zn and PC groups. Daily supplementation with Fe , Zn and FeZn benefitted only certain cognitive functions among female adolescents in the college setting. The results also support a beneficial effect of FeZn on depression in adolescent girls.**

***Index Terms*—Behavior, cognition, iron, zinc .**

I. Introduction

It is well established that deficiencies of both iron and zinc are highly prevalent in developing and developed countries [1]. The World Health Organization estimates that worldwide, 2 billion people are anemic and twice as many are iron deficient [2] With regard to zinc deficiency**,** an estimated 1.3 billion worldwide are at risk due to inadequate zinc intake [3] . Deficiencies of iron and zinc often coexist [4],[5] because iron and zinc are most bio available from many of the same foods and their absorption is inhibited by many of the same dietary substances [6].

Over the past three decades, there have been considerable numbers of studies on the cognitive and behavioral effects of iron and zinc deficiencies. A large body of research findings are consistent with the negative effect of lowered iron and zinc status on cognitive functions of infants and school children. Less research has focused on the cognitive effects of iron and zinc deficiencies and subsequently iron and zinc supplementation in adolescents or young adults [7], [8].

There is a also a general belief that compromised iron and zinc status may be associated with alterations in behavior such as anxiety, depression and fatigue. The few supplementation trials that were designed to test the efficacy of iron and zinc supplements in alleviating anxiety, depression and fatigue have yielded mixed results. [9] - [14]. Consistent information on the effect of iron and zinc on cognitive performance (mental speed, attention, verbal and visual memory as well as abstract reasoning) and certain behavioral measures (anxiety , depression and fatigue) especially among adolescent girls in the college setting are found lacking ; hence their benefits on these psychological functions remain speculative. This is of particular interest since adolescent girls are a highly vulnerable group due to inadequate intake of iron and zinc that fail to meet their high physiological requirements for growing body tissues, expanding red cell mass, and onset of menarche. In addition to these factors, certain non-dietary factors such as high menstrual losses, strenuous exercise, pregnancy, low socio-economic status and ethnicity may also increase their risk of both iron and zinc deficiencies [15]. Therefore it is likely that cognitive deficits and behavioral alterations associated with low iron and zinc nutriture is possible among female adolescents and could be improved with iron and zinc repletion. Hence, the present study was undertaken to examine the relative effects of iron, zinc and combined iron and zinc supplementation on certain cognitive functions and behavioral outcomes of female adolescents. The need to ascertain the benefits of combined iron and zinc supplementation as a logical strategy was felt since deficiencies of iron and zinc may coexist in vulnerable populations. Given the widespread prevalence of iron and zinc deficiencies among female adolescents, a documented report on the cognitive and behavioral effects of iron and zinc interventions would be relevant to the psychological functioning of a significant proportion of the world's population. Improving the psychological functions of

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adolescents is vital since it would enable them to cope with the demands and challenges of the present day academic and work environment. This paper describes the cognitive and behavioral response of female college students to four months of supplementation with iron, zinc or both

II. Methods

A. Subjects and Location

The study was conducted in Queen Mary's College, Chennai, run by the Government of Tamil Nadu. It is one of the oldest colleges catering to over 3000 students including 165 hostel residents belonging to low and middle income families. The subjects were adolescent girls, 17-19 y old, who met the following criteria: willing to participate in the study, healthy, residing in the hostel, had not taken multivitamin-mineral supplements and had not donated blood in the last 6months. Written informed consent was obtained from each of the subjects who were unaware of their entry into iron, zinc, iron and zinc and placebo groups. All procedures used in this study were reviewed and approved by the Doctoral Committee Board at the University of Madras, Chennai.

B. Study Design

This study was a randomized, double-blind, placebo-controlled intervention trial. This is a process whereby allocation of treatments is unknown to subjects, investigators and outcome assessors (i.e., double - blinding), and any subject being enrolled has an equal opportunity of being allocated to any one group (i.e.,randomization).Use of double blinding avoids bias to unintended interventions and ascertainment bias whereas use of randomization ensures that bias is prevented and that groups at baseline are comparable with respect to both measured and unmeasured variable [16]. The study was carried out with 109 subjects assigned at random to one of the four following groups:1) Ferrous fumarate (184.6 mg equivalent to 60 mg elemental iron), 2) zinc sulphate(82.4 mg equivalent to 30 mg elemental zinc), 3) Ferrous fumarate (184.6 mg equivalent to 60 mg elemental iron) along with zinc sulphate (82.4 mg equivalent to 30 mg elemental zinc) and placebo (lactose, 200mg and dicalcium phosphate 190mg). This dosage was based on the beneficial effects in iron and zinc status reported in earlier studies [17]-[19].

The treatments were randomly assigned in a double-blind manner using lottery method to the four treatment groups in the form of coded capsules to be taken as one capsule per day in between meals for a period of four months (Fig.1) All the capsules were manufactured by Alved Pharma & Foods Pvt. Ltd., Chennai as per standard specifications.. The capsules were packed in plastic containers, each containing 30 capsules and distributed at the beginning of each month. They were visited thrice a week to ensure intake of the capsules and were asked to fill out calendar forms every day covering a month to record their intake of the capsules and record side effects, if any. Compliance with supplementation was monitored on a monthly basis and the number of capsules remaining in each container was noted.. The formulations were kept by Alved Pharma & Foods Pvt. Ltd., Chennai in a sealed envelope until the trial ended. The sealed cover containing the details of the coded capsules was opened by the doctoral committee only at the conclusion of the study.

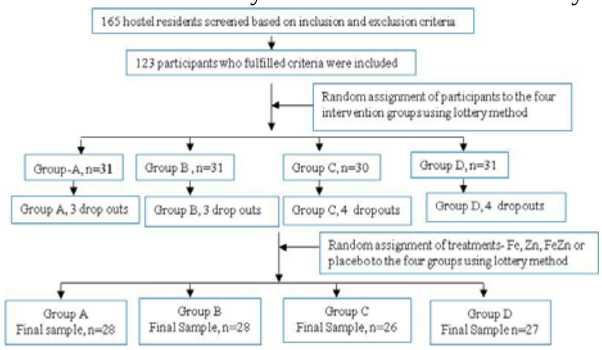

Fig. 1. Trial profile. allotment of participants and treatments to the four intervention groups

C. Measures

At entry, the subjects were administered a questionnaire to record socioeconomic information relating to their family size and type and per capita monthly income. Height and weight were also measured and Body Mass Index was determined by dividing a person's weight in kilograms (kg) by their height in meters (m) squared .Three day food records were also collected. The data obtained from the three day food recall inventory was converted into nutrient intake using the Nutritive Value of Indian Foods published by Indian Council Of Medical Research [20]. The adequacy of nutrient intake was then examined by drawing comparisons with the recommended daily allowance.

At base line and at the end of the intervention trial the assessment of hemoglobin, serum ferritin and serum zinc concentrations was done using standard laboratory procedures. For this purpose approximately 5ml of fasting venous blood samples were drawn from the subjects with the assistance of qualified medical /technical personnel The whole blood was used for hemoglobin estimation (Cyanometahemoglobin method) . The remaining venous blood was allowed to clot. The clotted blood was centrifuged and clear serum was collected. Serum was stored at 2°C to 8°C prior to testing and was used for the determination of ferritin (Enzyme Immunoassay ,Omega Diagnostic Ltd., Pathozyme-Ferritin Kit, FL, USA) and zinc (Colorimetric method ,Zinc Kit, Crest Biosystems, India) .

Seven cognitive functions and three aspects of behavior were also measured both at baseline and after 16 wk of treatment using standard procedures and instruments. The cognitive variables measured and the instruments used included the following: Mental speed-Digit Symbol Substitution Test[21], Sustained attention-Digit Vigilance test [22], Abstract reasoning- Standard Progressive Matrices [23], Immediate and delayed recall of verbal material, verbal recognition -Rey Auditory Verbal Learning Test[24], Immediate and delayed recall of visual material-Rey Complex Figure Test [25] and Recognition of visual material-PGI Memory Scale [26]. The behavioral variables measured and the instruments used included the following: State and trait anxiety- State and Trait Anxiety Inventory [27], Depression-Beck Depression Inventory [28], Perceived General Fatigue - Multidimensional Fatigue Inventory

[29] .These instruments were chosen as their ability to measure the selected psychological domains has been established in several researches in India . Also, their use in research and clinical practice in various situations and populations worldwide has been widely reported. After 16 wk the fasting venous blood was drawn and cognitive and behavioral tests were repeated.

D. Statistical Analyses

The data obtained in the study were subjected to descriptive analysis using mean and standard deviation. After testing the data for normality and homogeneity of variance, inter-group comparison on the effect of iron, zinc, and combined iron and zinc and placebo on certain cognitive and behavioral variables was done using ANCOVA. When the F-test result was significant, post hoc comparisons were performed with Bonferroni adjustment for multiple comparisons in a general linear model for ANCOVA. All analyses were adjusted for baseline performance on a given test. All statistical analyses were performed using SPSS version-10 .

III. Results

The 109 participants included in the study were between 17and 19 years of age, the mean age being 18.44. Based on the inclusion and exclusion criteria, the final sample consisted of 123 students who were selected out of 165 students by following the convenience sampling technique. Only 109 students completed the study. The subjects who dropped out of the study reported that they discontinued because they experienced side effects such as headache, hair fall, increased menstrual blood flow, giddiness and increased appetite. Fear of blood collection and sudden illness were the other reasons reported by the drop-outs. The compliance rate in all the four treatment groups was almost similar and ranged from 83%-86%. Majority of them (78%) were "intellectually average" based on Ravens Progressive Matrices test scores. Fifteen out of the 109 subjects (14%) were intellectually above average, 3 out of the 109 subjects (3%) were intellectually superior whereas 5 of them (5%) were intellectually below average.

Analysis of the nutrient intake of the subjects showed that their intake of energy, protein, carotene, riboflavin, thiamine, niacin, folic acid, ascorbic acid calcium, iron and zinc was below the Recommended Dietary Allowances [26] for Indian female adolescents (data not shown). The subjects under study consumed only 37% and 57% of the dietary iron and zinc respectively recommended for adolescents. Overall, 72% of them were non-vegetarians whereas only 28% were vegetarians. Based on Body Mass Index, 63 out of 109 (57%) subjects were of normal weight, 41 out of 109 (38%) were underweight, 5 out of 109 (5%) were overweight whereas none of them were obese.

Haemoglobin, serum ferritin and serum zinc were assessed at baseline and after 4 months of supplementation. At baseline 86 out of the 109 participants (79%) were iron deficient (serum ferritin levels <12 µg/L). Zinc deficiency (serum zinc concentrations <70µg/L) coexisted with iron deficiency in 30 out of the 86 participants (35%) with almost even number of cases in all four groups. After four months of supplementation, multiple comparisons of the four treatment groups showed that the mean hemoglobin concentrations improved, though not significantly in the *Fe* and *FeZn* groups when compared to the PC group. As expected, supplementation with iron alone or in combination with zinc resulted in significant improvement in the mean serum ferritin concentrations compared to the PC group (*Fe* vs *PC*, P<0.01, *FeZn* vs *PC* (P<0.05) . The *Zn* group had significantly higher mean serum zinc concentrations (P<0.01) than the Fe, *FeZn* and PC groups.

Regarding cognitive functions, after 4 months of intervention, Fe, Zn and *FeZn* had no significant effect on the speed component of the Digit symbol substitution test . The groups supplemented with *Fe*, *Zn* and *FeZn* performed significantly better than the *PC* group (P< 0.01) on the accuracy component of the Digit symbol substitution test as evidenced by the error scores obtained. Intergroup comparisons demonstrated no discernible differences in the *Fe*, *Zn* and *FeZn* group on the error scores. The *Fe*, Zn and *FeZn* groups improved in the time and error scores of the Digit Vigilance Test for sustained attention compared to the PC group, but the improvement in scores did not achieve significance. Similarly, *Fe*, *Zn* and *FeZn* groups improved, though not significantly in the immediate and delayed recall verbal test scores compared to the PC group. Also the trend towards improvement in the visual and verbal recognition test scores in the *Fe*, *Zn* and *FeZn* groups compared with the *PC* group failed to reach significance. The groups supplemented with Fe and *FeZn* scored higher on the cognitive function of visual memory (both in the immediate as well as in the delayed components of the test) than the PC group. These results support a beneficial effect of iron, zinc and both iron and zinc only on certain cognitive functions among female adolescents in the college setting (Table I and Table II).

The changes in anxiety, depression and general fatigue scores after 4 mo of supplementation with Fe, *Zn* or both *Fe* and *Zn* are shown in Table III. Supplementation with Fe, Zn and *FeZn* did not significantly decrease state and trait anxiety test scores of the *Fe*, *Zn* and *FeZn* groups compared to the PC group. Similarly *Fe*, *Zn* and *FeZn* did not change the perceived general fatigue scores in the treated groups compared to the PC group. However, at the end of the study combined *FeZn* supplementation significantly reduced the Beck Depression Inventory scores in *FeZn* group compared to the PC group (P< 0.05). Surprisingly, the depression scores were not significantly reduced in both the *Fe* alone and *Zn* alone groups

IV. Discussion

A. Influence of Iron and Zinc on Certain Aspects of Cognition

Cognition is a field-of-thought process by which an individual processes information through skills of perception, thinking, memory, learning and attention [30]. It also includes

TABLE I: MULTIPLE COMPARISONS AMONG THE TREATMENT GROUPS ON ADJUSTED POSTTEST SCORES ON COGNITIVE MEASURES.

	Baseline	Posttest	Adjusted posttest [8]
Mental Speed (Time component)			
Iron supplemented (Fe)	211.82±47.48 [7]	175.89±50.30	175.00±7.82 [1c,4c,6c]
Zinc supplemented Zn)	205.18±50.87	175.25±47.16	175.99±7.82 [2c,5c]
Iron and zinc supplemented (FeZn)	214.69±56.76	173.96±39.67	172.36±8.12 [3c]
Placebo controlled(PC)	201.30±57.47	183.07±32.94	184.77±7.98
Mental speed (error component)			
Iron supplemented	4.04±6.21	0.14±0.45	0.08±0.17 [1a,4c,6c]
Zinc supplemented	1.29±3.20	0.00±0.00	0.02±0.16 [2a,5c]
Iron and zinc supplemented	1.23±3.23	0.01±0.27	0.10±0.17 [3a]
Placebo controlled	1.63±2.96	0.89±1.65	0.90±0.17
Sustained attention (time component)			
Iron supplemented	423.18±90.27	373.43±76.19	382.00±13.23 [1c,4c,6c]
Zinc supplemented	447.32±90.17	368.75±63.24	367.79±13.13 [2c,5c]
Iron and zinc supplemented	466.15±102.26	350.42±92.13	342.03±13.72 [3c]
Placebo controlled	444.44±73.03	391.07±77.54	391.25±13.37
Sustained attention (error component)			
Iron supplemented	6.96±7.10	3.25±3.85	3.19±0.86 [1c,4c,6c]
Zinc supplemented	6.96±7.41	2.86±2.85	2.80±0.86 [2c,5c]
Iron and zinc supplemented	6.85±5.67	5.12±5.38	2.80±0.86 [3c]
Placebo controlled	6.26±6.42	5.59±6.59	5.72±0.88
Abstract reasoning			
Iron supplemented	37.82±10.84	42.14±10.43	42.52±1.45 [1c,4c,6c]
Zinc supplemented	38.68±10.44	40.64±11.97	40.46±1.45 [2c,5c]
Iron and zinc supplemented	39.62±10.15	43.77±8.73	42.97±1.51 [3c]
Placebo Controlled	37.56±11.02	41.07±9.77	41.62±1.48

Notes for Tables I-III:

Iron supplemented: *n*=28, 60mg elemental iron; zinc supplemented: *n*=28, 30mg elemental zinc, Iron and zinc supplemented : *n*=26,60mg elemental iron+ 30 mg elemental zinc, placebo, *n* =27.Values in the row with different superscript letters and numbers show differences between groups in adjusted posttest scores.

1 Fe *vs* PC, 2 Zn vs PC, 3 FeZn vs PC, 4 Fe vs FeZn, 5 Zn vs Fe Zn, 6 Fe vs Zn;

7 Mean ±SE, 8 Adjusted for baseline performance on a given test

a Significant differences between groups ,P< 0.01 level,

b Significant differences between groups , P< 0.05 level,

c No Significant differences between groups

TABLE II: MULTIPLE COMPARISONS AMONG THE TREATMENT GROUPS ON ADJUSTED POSTTEST SCORES ON COGNITIVE MEASURES- VERBAL MEMORY AND RECOGNITION, VISUAL MEMORY AND RECOGNITION.

	Baseline	Posttest	Adjusted posttest [8]
Verbal memory (immediate recall)			
Iron supplemented	11.82±2.92 [7]	13.61±2.35	13.41±0.41 [1c,4c,6c]
Zinc supplemented	11.18±2.37	12.68±2.44	12.70±0.41 [2c,5c]
Iron and zinc supplemented	10.85±2.81	13.35±1.94	13.48±0.43 [3c]
Placebo Controlled	11.11±3.71	12.30±2.77	12.34±0.42
Verbal memory (delayed recall)			
Iron supplemented	12.25±2.78	13.43±2.15	13.32±0.38 [1c,4c,6c]
Zinc supplemented	11.46±2.62	12.54±2.73	12.72±0.38 [2c,5c]
Iron and zinc supplemented	11.65±2.67	13.35±1.77	13.38±0.39 [3c]
Placebo Controlled	11.85±3.05	12.63±2.15	12.61±0.38
Verbal recognition			
Iron	14.04±1.71	14.86±0.59	14.82±0.15 [1c,4c,6c]
Zinc	13.71±1.63	14.64±1.06	14.64±0.15 [2c,5c]
Iron and zinc supplemented	13.62±2.17	14.73±0.67	14.74±0.16 [6c]
Placebo Controlled	13.59±1.78	14.59±0.89	14.61±0.15
Visual memory (immediate recall)			
Iron supplemented	30.00±4.13	34.00±1.36	34.16±0.44 [1a 4c 6c]
Zinc supplemented	31.82±3.91	32.93±2.55	32.59±0.44 [2c 5c]
Iron and zinc supplemented	29.92±3.93	32.58±3.21	32.76±0.45 [1b]
Placebo Controlled	30.63±3.40	31.82±2.66	31.80±0.44
Visual memory (Delayed recall)			
Iron supplemented	30.29±4.20	33.50±1.73	33.64±0.46 [1b 4c 6c]
Zinc supplemented	31.14±4.13	32.71±2.65	32.68±0.45 [2c 5c]
Iron and zinc supplemented	30.73±3.87	32.73±3.12	32.78±0.47 [3b]
Placebo Controlled	31.74±3.08	31.11±2.41	30.96±0.47
Visual recognition			
Iron supplemented	9.14±1.04	9.79±0.57	9.82±0.08 [1c,4c,6c]
Zinc supplemented	9.50±0.79	9.86±0.36	9.83±0.08 [2c,5c]
Iron and zinc supplemented	9.46±0.71	9.77±0.59	9.74±0.08 [6c]
Placebo Controlled	9.30±0.95	9.96±0.19	9.17±0.08

Skilled behaviors and response execution [31]. Several studies among preschool and school aged children and a few studies among older adolescents and adults showed that iron and zinc can benefit cognitive functions such as speed of information processing, attention, reasoning, learning and memory [7],[8],[18], [32].

In this study, the effect of iron and zinc on the cognitive function of mental speed was measured based on the error and time component of the Digit Symbol Substitution Test. The decrease in the number of errors committed by the groups supplemented with Fe, Zn and FeZn compared to the PC group reflects the beneficial effect of these micronutrients in improving accuracy levels of the subjects. The lack of discernible difference among the Fe, Zn and FeZn in the accuracy component (error score) of the test suggests that Fe and Zn either as single supplements or in the combined form are equally effective in improving concentration . It is quite likely that the improvement in iron and zinc status of the adolescent girls after supplementation may have mediated the favorable response observed. This view is supported by

TABLE III: MULTIPLE COMPARISONS AMONG THE TREATMENT GROUPS ON ADJUSTED POSTTEST SCORES ON BEHAVIORAL OUTCOMES –STATE ANXIETY, TRAIT ANXIETY, DEPRESSION AND PERCEIVED GENERAL FATIGUE.

	Baseline	Posttest	Adjusted posttest [8]
State anxiety			
Iron supplemented (Fe)	45.07±11.27 [7]	43.25±11.10	42.91±2.02 [1c,4c,6c]
Zinc supplemented Zn)	41.71±9.78	41.43±10.08	42.35±2.03 [2c,5c]
Iron and zinc supplemented (FeZn)	51.08±9.47	45.88±12.38	43.26±2.21 [3c]
Placebo controlled(PC)	39.11±11.30	44.70±11.80	46.61±2.21
Trait anxiety			
Iron supplemented	51.68±11.11	49.54±11.32	47.79. ±2.09 [1c,4c,6c]
Zinc supplemented	47.54±10.34	48.25±12.50	48.39±2.05 [2c,5c]
Iron and zinc supplemented	44.31±10.04	41.77±12.71	43.37±2.16 [3c]
Placebo controlled	47.63±9.71	49.07±10.47	49.18±2.09
Depression			
Iron supplemented	17.58±7.43	16.81±9.83	17.56±1.34 [1c,4c,6c]
Zinc supplemented	17.39±7.38	16.32±9.76	17.4379±1.44 [2c,5c]
Iron and zinc supplemented	20.81±7.48	13.62±7.72	12.513±1.49 [3b]
Placebo controlled	17.74±8.47	17.26±9.46	18.14±1.46
Perceived General fatigue			
Iron supplemented	12.50±3.66	10.82±3.53	10.84±0.61 [1c,4c,6c]
Zinc supplemented	12.50±3.16	11.29±3.22	11.30±0.61 [2c,5c]
Iron and zinc supplemented	13.12±2.76	10.69±2.90	10.54±0.63 [3c]
Placebo controlled	12.15±2.63	12.37±3.51	12.48±0.62

Earlier studies that have demonstrated strong association of iron status and zinc status with cognitive functioning [7], [8] [18], [34],[35]. By contrast to previous studies, the groups supplemented with Fe and *Zn* and *FeZn* did not show any improvement in the time component of the test (mental speed) compared to the PC group [2],[7],[8],[18],[32].This could be due to the fact that the subjects under study may have paid more attention to the accuracy component of the test rather than the speed component of the same test. Perhaps the use of instruments that exclusively measure speed might have yielded definitive results on the potential role of iron and zinc supplements on the cognitive function of mental speed. In a study by Murray-Kolb and Beard [2] four months of iron treatment was provided to young women between 18-35 years of age at Pennsylvania State University. The improvement in hemoglobin concentrations was related to improved speed in completing the cognitive test. In the present study there was no significant improvement in hemoglobin concentrations in the Fe, Zn and FeZn groups. Taken together, the results suggest that hemoglobin concentrations and severity of anemia may affect mental speed.

Contrary to expectation, supplementation with Fe, Zn and *FeZn* did not have a beneficial effect of the cognitive function of attention. However, other supplementation studies reported improvement in attention with iron supplementation among young women [2] and adolescents [33]. Similarly, the lack of significant improvement in sustained attention with zinc supplements stands in contrast with the findings of Penland *et al***.,** [32] who reported improvement in attention tasks in the group supplemented with zinc. The present finding could possibly be attributed to the difference in the nature of the instrument that was used in this study for measuring sustained attention compared to the computerized tests used in the earlier studies. It may be noted that the Digit Vigilance test which was used in the present study demands high level of mental effort and attention over a longer period. It is quite likely that mental fatigue, eye strain, boredom, lack of motivation could have also influenced the outcomes of the test [34].

The favorable improvement in visual memory in the *Fe* group confirms the results of previous studies that demonstrated the beneficial effect of *Fe* on the cognitive function of visual memory [19], [35]. Perhaps the marked increase in serum ferritin concentrations in the Fe group and *FeZn* groups may have mediated improvement in the cognitive function of visual memory. This speculation is further supported by the lack of improvement observed in the *Zn* group among whom there was no significant improvement in serum ferritin levels. Hence, it is quite possible that the lowered serum ferritin levels in this group may have hindered the potential benefits of zinc from being fully expressed. Based on these findings it appears that the cognitive function of visual memory is influenced more by iron status than zinc status. The findings also show that Fe and *FeZn* supplements are more beneficial in improving the cognitive function of immediate and delayed recall of visual memory than *Zn*.

The modest improvement in other cognitive functions such as abstract reasoning, immediate and delayed recall of verbal material and verbal as well as visual recognition in the *Fe*, *Zn* and *Fe Zn* groups do not demonstrate convincingly that iron and zinc could meaningfully improve overall cognitive performance. The lack of significant improvement in the cognitive functions of delayed recall and recognition of verbal material in the iron supplemented group is consistent with the finding of Bruner *et al.,* [36]. Similarly, the lack of improvement in verbal memory in the zinc supplemented group is in accordance with the findings of Darnell and Sandstead [19]. However, the findings stand in contrast to earlier studies that demonstrated significant improvement in the cognitive function of immediate recall of verbal material and abstract reasoning with iron supplementation[9],[19] [36]. Also, the results observed in the zinc supplemented group, failed to support the findings of other researchers who demonstrated significant improvement in reasoning ability and recognition of visual and verbal material among those who were treated with zinc (8,18,).

Several biological mechanisms potentially link deficiencies of iron and zinc with impaired cognitive performance. Iron deficiency results in decreased iron stores, including decreased iron in the central nervous system, even before red blood cell production is affected. Disordered cerebral oxidative metabolism attributable to low levels of heme- containing and iron-dependent enzymes result in behavioral abnormalities in animals. Furthermore, alterations in metabolism of several putative neurotransmitters have been described in both iron deficient animal and humans [37)]. Indirectly, iron deficiency could affect cognitive and behavior by reducing an individual's attentiveness and responsiveness to the environment. Iron deficient individuals suffer from malaise, are lethargic, and may be less vigilant in performing tasks and this may lead to fewer learning experiences and less ability to acquire information [9], [38]. Zinc deficiency may affect cognitive development by alterations in attention, activity, neuropsychological behavior and motor development. The exact mechanisms are not clear but it appears that zinc is essential for neurogenesis, neuronal migration, synaptogenesis and its deficiency could interfere with neurotransmission and subsequent neuropsychological behavior [30]. Further, Bryan *et al.* [39] reported that zinc may indirectly affect cognitive performance through its interaction with other micronutrients, especially iron.

In this study the lack of significant improvement in some of the cognitive functions such attention reasoning, verbal memory, verbal and visual recognition that were assessed may be attributed to the influence of the following factors: Firstly, the iron status of most of the participants in all four treatment groups was poor before supplementation as evidenced by their low serum ferritin concentrations (serum ferritin concentrations <12µg/L). Similarly low zinc status (serum zinc concentrations <70µg/L) was also observed among many of them. It is quite possible that lowered iron and zinc status observed among the participants before supplementation may have exerted a cumulative effect over time on cognitive functioning which may not have facilitated a distinct and significant improvement in all cognitive domains with supplementation over a period of four months

Secondly, the period of supplementation (4 months) in the present study may not have been adequate enough to facilitate favorable increments in brain iron and zinc pools for substantial enhancement in all the cognitive functions that were assessed. Further, the lack of significant improvement in cognitive functions in this study could be attributed to the possibility of other coexisting micronutrient deficiencies such folate, riboflavin, vitamin C and vitamin B-12 in the population studied. This reason is supported by the findings of Goodwin *et al*.,[40)] who found significant correlation between biochemical indices of vitamin status and cognitive functioning. They reported significant correlation between folate, riboflavin, vitamin C and vitamin B-12 status and memory capacity. The levels of riboflavin and folate were also found to be positively related to the ability to think abstractly. Besides, other double- blind placebo-controlled studies reported, that multivitamin and mineral supplementation are associated with better moods, memory, attention and scores of intelligence [41]-[43]. In fact, a few researchers also demonstrated that the benefits of *Fe* and *Zn* supplements on cognitive functions are further amplified with the addition of other limiting micronutrients [8], [18]. Based on these observations, it is quite possible that the inclusion of a multiple micronutrient supplement along with iron and zinc could have yielded more favorable results among the adolescent population in the present study.

B. Influence of Iron and Zinc on Certain Aspects of Behavior

In this study, the state anxiety and trait anxiety scores of the *Fe*, *Zn* and *FeZn* groups did not significantly differ from the scores obtained by the PC group after intervention. The lack of significant decrease in state and trait anxiety scores may be attributed to certain nutritional and non-nutritional factors that could have masked or overpowered the beneficial effect of iron and zinc supplements in reducing anxiety among female adolescents.

It is well known that deficiencies of several micronutrients other than *Fe* and *Zn* can cause anxiety. For example, B-vitamins are known to be crucial for thousand of metabolic reactions in the body including those of the central nervous system and brain. Vitamin B-6 and Vitamin B-1, in particular, are necessary for the production of Gamma Amino Butyric Acid (GABA) which is supposed to be the main calming neurotransmitter of the brain. Vitamin B-6 is also important in the production of neurotransmitters such as serotonin, dopamine and nor-epinephrine, an imbalance of which has been implicated in anxiety [44]. Further, Prousky, [45] reported that niacinamide is easily used by brain cells and is effective for treatment of anxiety. Besides, magnesium, calcium and multivitamins have also been reported to be essential for reducing anxiety and also for general brain health and function [46], [47].In view of these facts, it appears that the lack of significant decrease in state and trait anxiety may be due to the likelihood of multiple micronutrient deficiencies in the population studied.

Although nutritional factors have been reported to alleviate anxiety, it may be noted that generally, state anxiety can be reduced by techniques such as relaxation, physical exercise and yoga. Trait anxiety, being a personality predisposition, is difficult to change and modify within a short span of time. Hence it appears that continuous practice of the above mentioned techniques besides improved iron and zinc status *per se* may prove beneficial in overcoming anxiety among female adolescents. Further, it is quite possible that other non-nutritional factors such as general stress, stress related to studying and writing examinations, grade competition, fear of the future with specific reference to job prospects and marriage could also influence anxiety levels among female adolescents. Hence, the lack of significant change in the behavioral aspect of state and trait anxiety despite significant improvement in serum ferritin and serum zinc concentrations in the *Fe* and *Zn* groups respectively may be attributed to the above mentioned factors.The methodological limitations of the study need to be considered . State and Trait Anxiety Inventory [29] which is a self reported questionnaire aimed at assessing the levels of anxiety at the moment (State anxiety) and in general (trait anxiety) was the only measure used in the study. Perhaps the use of more objective assessment of anxiety on physiological parameters could have yielded more conclusive results on the potential role of iron and zinc in alleviating anxiety in female adolescents.

After supplementation, the results revealed no significant decrease in depression scores in the groups supplemented with iron and zinc compared to that in the PC group. The lack of significant decrease in depression levels among female adolescents selected for the study, despite significant improvement in their serum ferritin and serum zinc concentrations after supplementation with Fe and *Zn* respectively, raises the possibility that depression among adolescents may also be linked to other factors besides iron and zinc status *per se.*

Perhaps, factors such as low self esteem, poor family relationship, poor social support, high control by teachers, high competition among classmates, less scope for participation, more pressure to achieve, low peer acceptance and stressful events among adolescents may contribute to more depression symptoms among adolescents than iron and zinc status alone. However, a novel observation in the present study is the significant decline in depression scores in the *FeZn* group. The beneficial effect observed in this group may be attributed to the fact that the subjects in this group had moderate levels of depression (20.81) at baseline compared to the groups treated with Fe and Zn alone who were found to have mild levels of depression. Hence, it is likely that the benefits of iron and zinc are more profound among individuals with higher levels of depression.

This study demonstrated there was no difference in the level of perceived general fatigue between the group supplemented with Fe and the PC group. The lack of a treatment effect with Fe supplementation was an unexpected outcome and is not in consonance with the findings of Verdon *et al.*, [10] and Patterson *et al*., [11]. Similarly, the group supplemented with Zn and Fe Zn and the PC group did not differ in their level of perceived general fatigue**.**

The lack of significant decrease in perceived fatigue levels after supplementation with Fe an Zn either alone or in the combined form may be attributed to the nature of instrument used (General fatigue subscale of the Multi Dimensional Fatigue Inventory) in the present study. It must be noted that this is a self-reported questionnaire and is a subjective measure of fatigue. Perhaps the use of objective assessments that measure physiological parameters related to fatigue could have yielded definitive results on the potential role of iron and zinc in reducing fatigue. Another possible explanation is the likelihood of other vitamin and mineral deficiencies among the participants such as deficiencies of vitamin B-1, vitamin B-6, vitamin B-12, magnesium and potassium which are known to be associated with increased fatigue [48],[49].

V. Conclusions

Daily supplementation with iron, zinc and combined iron and zinc were efficacious in improving the cognitive function of mental speed on accuracy levels. Iron and combined iron and zinc were both efficacious in improving the cognitive function of visual memory. Combined iron and zinc supplementation was effective in reducing depression among female adolescent compared to single supplements of the same. This study suggests that supplementation with 60 mg elemental iron, 30 mg elemental zinc and both 60 mg elemental iron and 30 mg elemental zinc combined for a period of four months improves certain cognitive functions among female adolescents. Precise mechanisms by which iron and zinc influence cognitive functions and behavior requires further investigation. Cognitive and behavioral response to iron and zinc supplements with or without other micronutrients should be assessed in this and other locations were multiple micronutrient deficiencies exist for better expression of cognitive potential and behavior in vulnerable populations.

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