

## Evaluation of Iron Supplementation Effects on Various Serum Minerals in Pregnant Anaemic Patients of Sargodha Region

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### Abstract

Pregnant anaemic patients of Sargodha region were asked to take oral iron supplementation (150 mg per day) in the form of available medicines up to the period of two months. Blood samples were collected before the initiation of treatment and secondly after the two months of treatment. Evaluation of serum minerals shows that, iron level increases from 65 µg/dl-75 µg/dl in Group-1 and 62 µg/dl-78 µg/dl in Group-2 before and after supplementation respectively this increase is statistically significant ( $p=0.000$ ). Results shows that iron supplementations have no effect on serum calcium. So values of serum calcium remain same after two months in both groups. Change in P values are statistically insignificant ( $P>0.05$ ). The mean values of serum calcium remains same in Group-1 is 8.9-9.1 mg/dl ( $p=0.07$ ),  $SD=0.66-0.7$  and in Group-2 it is 9.0-9.1 mg/dl ( $p=0.83$ ),  $SD=0.6-0.7$  before and after supplementation respectively and values of serum copper decreases in both groups. Change in P values are statistically insignificant ( $P>0.05$ ). The mean values of serum Cu in Group-1 is 108-107 µg/dl ( $p=0.35$ ),  $SD=28.3-28.0$  and in Group-2 it is 110-109 µg/dl ( $p=0.13$ ),  $SD=24.4-23.0$  before and after supplementation respectively. Serum zinc values significantly decrease ( $p=0.00$ ) from 68 µg/dl - 66 µg/dl and 68 µg/dl - 67 µg/dl in Group-1 and Group-2 respectively. It may be due to positive effect on serum iron level and negative effect on serum zinc status. Serum calcium and copper remains unaffected from iron supplementation. The variations were almost similar in both age groups (1 & 2) means effects of iron supplementations are similar irrespective of the age of pregnant anaemic patients, the only noticeable change was that variations are comparatively fast in Group-1 as compare to Group-2 it may because of greater absorption of nutrients in young ones than that of aged subjects.

**Keywords:** Iron supplementation; Serum; Sargodha region; Calcium; Copper

### Introduction

Pregnancy is associated with physiological changes that results in increased plasma volume and red blood cells and leads to the reduction in concentrations of circulating nutrient-binding proteins and micronutrients (for examples, iron, folic acid, and vitamin B12). These changes which occur in the body physiology become associated with malnutrition in many developing countries of the world, leading to micronutrient deficiency states, such as anaemia. Pregnancy is a time period in which requirement of iron is greater than normal as compare to absorbable iron intake hence iron deficiency takes place which leads to anaemia. Most of the women start their pregnancy with moderately or completely depleted iron reserves and the severity of the anaemia is correlated with the amount of iron reserves [1]. Pregnancy is the time when, there is a greater demand for iron to meet the requirement of increasing red blood cells and other quickly developing cells mass expansion in the mother. The fetal and placental blood and blood loss at delivery is another indication for providing extra iron in the second and third trimester of pregnancy some times in association with folic acid. This is provoked by poor absorption of iron due to unpleasant effects of pregnancy on the gastrointestinal tract, which include nausea and vomiting and some other motility disorder like indigestion [2]. In developed countries the occurrence of anaemia during pregnancy is about 20%. Pregnancy causes a lot of physiological and hormonal changes. Increase in plasma is more dramatic than red blood cell mass expansion therefore it causes erythropoiesis, the concentration of haemoglobin, the erythrocyte count and the haematocrit decrease during normal pregnancy.

Nutrient demand increase during the period of pregnancy to

support fetal growth and maternal health. Iron requirements of pregnant women are approximately double than that of non-pregnant women because of increased blood volume during pregnancy, increased needs of the fetus, and loses of blood that occur during delivery [3]. Such increasing demands leads to a decline in iron stores during pregnancy and eventually can produce iron-deficient erythropoiesis and anemia because it become difficult to achieve a neutral iron balance. In developing countries the occurrence of iron deficiency in pregnant women is about 30-40% [4]. Iron deficiency anemia has a negative effect on fetus mental development and behavior and it is also concerned with adult cardiovascular diseases [5]. Many studies provides evidences that maternal iron deficiency is responsible of preterm delivery, low birth weight [6], and possibly placental Anaemia is a main causative factor of maternal mortality in underdeveloped countries [7]. In some Asian countries like Indonesia and India high frequency of iron deficiency was also reported in pregnancy along with maternal and fetal loss. In the same way it is associated with high prenatal mortality rate [8]. During pregnancy body absorbs iron more effectively. Iron will also helpful to prevent the symptoms of tiredness,

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weakness, irritability, and depression. A study that was conducted in India shows a positive relationship between iron supplementations and birth outcomes. Pregnant women commonly develop iron deficiency anaemia because of increasing iron demand of the developing fetus and placenta and the increased volume of blood circulating in the body of women during pregnancy Auerbach et al. [9].

Large number of mortalities during pregnancy (e.g premature deliveries and infants with low birth weight) is because of iron deficiencies. The worldwide anaemia prevalence data describes that normal dietary intakes of iron are insufficient to meet up reserves and the infant may be weaned with a larger iron store. The extensive use of iron supplementation makes interactions between iron and other micronutrients of special nutritional relevance. Some recent clinical studies propose that iron absorption from prenatal supplements is unfavorably affected by other minerals such as calcium and that calcium supplementation has negative effect (e.g decrease) the absorption of dietary iron. If combined iron and calcium supplementation is required no adverse effects on iron absorption will occur. If taken with meals calcium supplements used regularly may make it more difficult to meet daily iron requirements [10]. Iron can interact with intestinal absorption of bivalent metals such as copper [11]. In pregnant women depletion of Plasma zinc concentration begin in the start of pregnancy. The depletion in the zinc levels is due to haemodilution, decrease in levels of zinc binding protein, hormonal changes during pregnancy and active transport of zinc from the mother to the fetus. Single meal studies have demonstrated that intake of supplemental iron (in liquid form) the absorption of zinc was reduced in a dose-dependent manner. However, when iron was added to solid food no effect was found on zinc absorption in adults [12].

## Materials and Methods

Our subjects were 40 pregnant women having Hb (>11g/dL) and having age 20-45 years selected from Sargodha region. The haematological parameters used to investigate anaemia in pregnant women of Sargodha region were haemoglobin concentration and haematocrit value. The normal haemoglobin and the normal haematocrit cutoff levels for pregnant women used in this study are Hb  $\geq$  11g/dl and Hct  $\geq$  33%, respectively [4]. To assess the iron status of subject's serum iron was used. Pregnant women up to 30 years were categorized as Group-1 and pregnant women who were of more than 30 years were categorized as Group-2. All women were asked to use iron tablets (150 mg per day) in order to enhance their serum iron level for a period of two months in the form of available branded medicines and assess its effects on serum minerals level in pregnant anaemic women of Sargodha region. Blood sample of pregnant women was collected at start (before the initiation of treatment) than after two months of treatment. Study of blood serum was done to find out changes which occur in the level of serum Fe, serum Cu, serum Ca and serum Zn levels.

## Chemicals used

Hydrogen peroxide, Nitric acid, Distilled water, Deionized water, Salts of iron, Ca, Cu and Zn serum samples.

## Method

Evaluation of serum Fe, Ca, Cu and Zn was done. For this purpose digestion of serum was done. 2 ml of serum was mixed with 1.5 ml of nitric acid and allow it to stay for one night. Than its heating was done upto 280°C along with continuous stirring and addition of hydrogen peroxide was done (drope wise) even sample became clear. Standard

solutions of iron, Ca, Cu and Zn were prepared (by using their salts) for drawing calibration curves on spectrophotometer. Than samples were run in atomic absorption spectrophotometer the absorbance of the samples was measured which tells the concentration of serum minerals.

## Results

Results were evaluated by statistical analysis. The Statistical analysis used in present study included paired sample t-test to compare means. P values obtained to find out the significance of each parameter. Means, median, mode, range and standard deviations (SD), were calculated for each haematological. All data was analyzed by using the SPSS program (SPSS Inc. Statistical Package for Social Science, Version 16, SPSS Inc, USA).

### Serum Iron (Fe)

Results shows that iron supplementations have a significant effect on serum iron level. So values rises significantly after supplementation in both groups. Change in P values is statistically significant ( $P < 0.05$ ). The mean values of serum iron rises in Group-1 from 62  $\mu\text{g/dl}$  to 75  $\mu\text{g/dl}$  ( $p=0.00$ ),  $SD=4.3-6.0$  and in Group-2 it is 62  $\mu\text{g/dl}$  -78  $\mu\text{g/dl}$  ( $p=0.00$ ),  $SD=1.8-3.6$  before and after supplementation respectively. Figure 1a and 1b shows a statistically significant increase in serum iron status after iron supplementations. Increasing trend is almost similar in both age groups, increase is very prominent.

### Serum calcium (Ca)

Results in Table 1 shows that iron supplementations have no effect

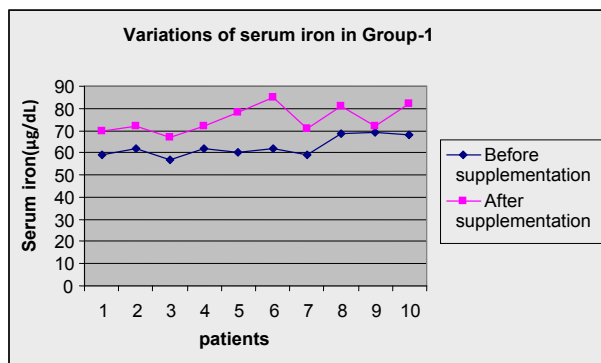


Figure 1a: Statistically significant increase in serum iron.

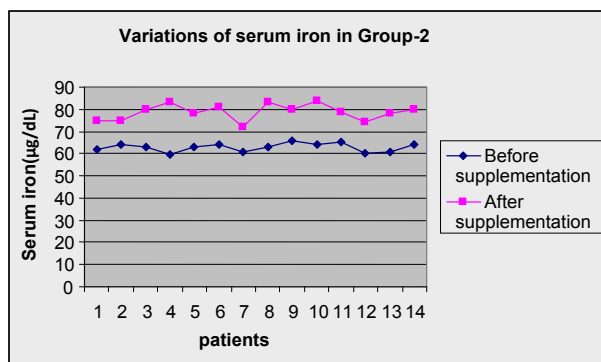


Figure 1b: Statistically significant increase in serum iron.

on serum calcium. So values remain same after two months in both groups. Change in P values are statistically insignificant ( $P>0.05$ ). The mean values of serum calcium remains same in Group-1 is 8.9-9.1 mg/dl ( $p=0.07$ ),  $SD=0.66-0.7$  and in Group-2 it is 9.0-9.1 mg/dl ( $p=0.83$ ),  $SD=0.6-0.7$  before and after supplementation respectively. Figure 1c and 1d shows that serum calcium level is exactly same after iron supplementations in both groups. Overall value of calcium is less because due to greater demand of Ca in pregnancy. Serum Ca was comparatively low when sample was collected before supplementation and it was found that its concentration remains unchanged after iron supplementations treatment. These results are in accordance to the previous studies which reveals that iron has no effect on serum calcium [13].

### Serum Copper (Cu)

Results show that values of serum copper decreases in both groups.

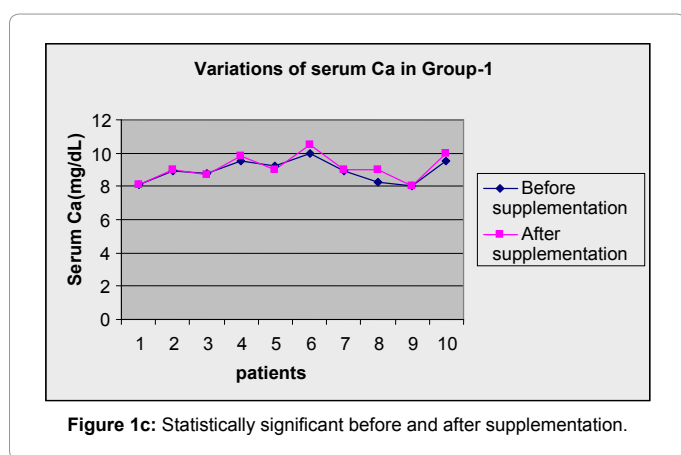


Figure 1c: Statistically significant before and after supplementation.

Change in P values are statistically insignificant ( $P>0.05$ ). The mean values of serum Cu in Group-1 is 108-107  $\mu\text{g/dl}$  ( $p=0.35$ ),  $SD=28.3-28.0$  and in Group-2 it is 110-109  $\mu\text{g/dl}$  ( $p=0.13$ ),  $SD=24.4-23.0$  before and after supplementation respectively.

Figure 2a and 2b shows that serum Cu level decreases after iron supplementation but not significantly. This reveals that iron can interact with serum copper and can decrease its concentration but not up to a significant level. Similar trend discussed in many studies that there is an opposite relation between the concentration of haemoglobin and serum copper level. So as during pregnancy with anaemia Hb decreases and copper increases but as iron increases Hb it decreases copper level. Copper and Hb levels are inversely correlated. This correlation is more significant in anaemic pregnant women [14].

### Serum Zinc (Zn)

Results in Table 2 shows that iron supplementations have a

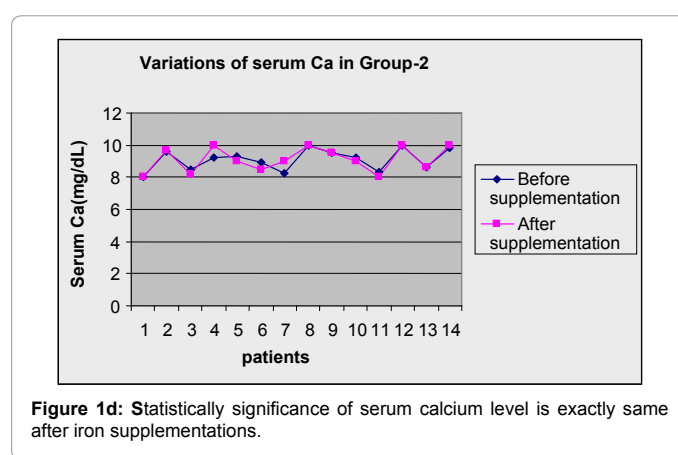


Figure 1d: Statistically significance of serum calcium level is exactly same after iron supplementations.

Statistical parameters	Serum Iron(Fe) ( $\mu\text{g/dl}$ )				Serum calcium(Ca) mg/dl			
	Group-1		Group-2		Group-1		Group-2	
	Before	After	Before	After	Before	After	Before	After
Mean	62.6500	75.0000	62.8500	78.7000	8.9140	9.1100	9.0864	9.1071
Median	62.0000	72.0000	63.0000	79.5000	8.9000	9.0000	9.2000	9.0000
Mode	62.00	72.00	64.00	80.00	8.90	9.00	9.20	10.00
Range	12.00	18.00	6.10	12.00	2.00	2.50	2.00	2.00
S.D	4.34645	6.01849	1.84589	3.64143	0.66106	0.79366	0.67107	0.76607
T value	-7.351		-16.950		-1.990		-0.210	
P value	0.000**		0.000**		0.078*		0.837*	

\*\* $P<0.05$  shows a significant change

Table 1: Iron supplementations have no effect on serum calcium.

Statistical parameters	Serum Copper(Cu)( $\mu\text{g/dl}$ )				Serum Zinc(Zn)( $\mu\text{g/dl}$ )			
	Group-1		Group-2		Group-1		Group-2	
	Before	After	Before	After	Before	After	Before	After
Mean	108.7000	107.8000	110.1429	109.2857	68.4000	66.1000	68.4286	67.0000
Median	103.0000	105.5000	114.0000	115.0000	67.5000	64.5000	68.5000	67.0000
Mode	82.00	81.00	79.00	78.00	62.00	70.00	65.00	67.00
Range	71.00	70.00	72.00	70.00	14.00	14.00	11.00	12.00
S.D	28.34725	28.42456	24.4221	23.92755	5.05964	4.97661	3.43543	3.44182
T value	0.987		1.578		10.776		3.150	
P value	0.350*		0.139*		0.000**		0.008**	

\* $P>0.05$  shows an insignificant change

\*\* $P<0.05$  shows a significant change

Table 2: Iron supplementations have a significant effect on serum Zn level.

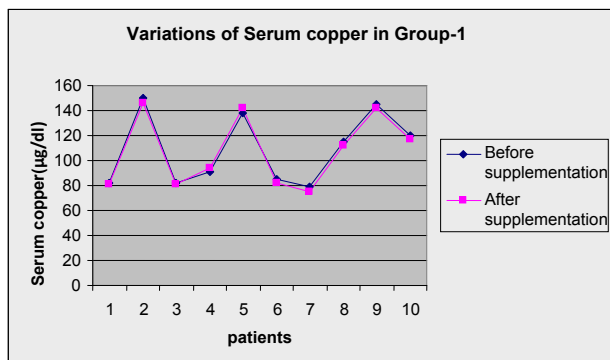


Figure 2a: Serum Cu level decreases after iron supplementation.

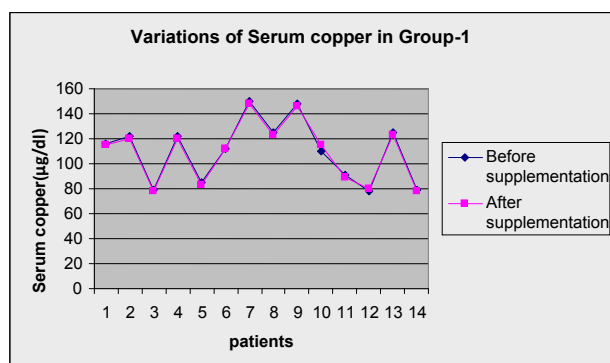


Figure 2b: Serum Cu level decreases after iron supplementation.

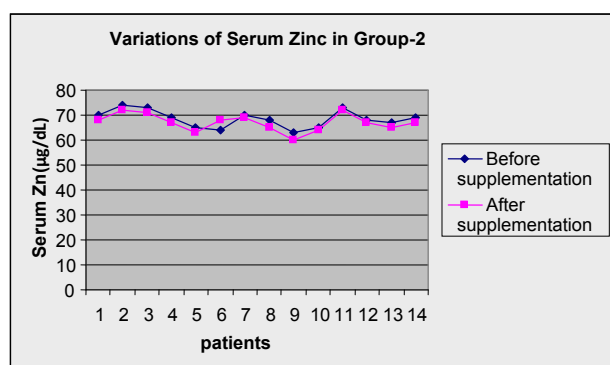


Figure 2c: Decrease in serum zinc status after taking oral iron supplementation.

significant effect on serum Zn level as its values significantly decreased. So values decreases significantly after one month in both groups. Change in P values is statistically significant ( $P < 0.05$ ). The mean values of serum iron decreases in Group-1 is 68-66 µg/dl ( $p=0.00$ ),  $SD=5.0-4.9$  and in Group-2 it is 68-67 µg/dl ( $p=0.00$ ),  $SD=3.4-3.4$  before and after supplementation respectively. Figure 2c shows a decrease in serum zinc status after taking oral iron supplementation. These results show a negative correlation between iron and serum zinc status.

## Discussion

Sejeny et al. [15], found a decreasing trend in serum zinc level

in a study conducted on pregnant anaemic patients of China. This study reveals a positive relation between serum iron and zinc level a decreasing trend in hemoglobin causes a decrease in serum zinc values. This positive correlation of haemoglobin concentrations with zinc and iron indicates that deficiencies of the two minerals were frequent and harsher in anaemic pregnant women. This deficiency during pregnancy is due to the deficiency of iron and zinc which may result from an expanded blood volume. This study also provides an evidence about the increasing demand of iron and zinc in pregnant anaemic patients [14].

These values change in anaemic condition as present study shows low value of iron when blood sample was collected at zero day (mean=62.6 µg/dl, and 62.8 µg/dl and  $SD=12$  and 6.0 in Group 1 and 2 respectively). As patients in present study suffering from anaemia due to which the values of serum iron are low in the same way it was noted that level of serum Zn was also low when blood sample was collected before supplementations (mean=68.4 µg/dl and 68.4 µg/dl and  $SD=5.0$  and in group-1 and 2 respectively). Level of serum Cu was in normal range when sample collected at zero day (before supplementation). Results shows a positive effect of iron supplementation on serum iron level which increases significantly ( $p=0.00$ ). While these medicines have a negative influence on serum Zinc status which decrease significantly ( $p=0.00$ ). Iron deficiency during pregnancy is common in Pakistan and has severe short and long term consequences such as fetal growth retardation and cardiovascular problems in the adult offspring's.

Hambidge et al. [13], reported the decrease in serum Zn level after iron supplementation (60 mg/day) and similar depletion trend in serum Zinc was found in pregnant teenagers after iron therapy (18 mg per day).

## Conclusion

The restrictions of serum zinc as a symbol of body zinc status make it complicated to distinguish under what physiological conditions negative effects of a high iron intake on zinc absorption are most likely to occur. Similar to the iron-zinc interaction zinc in high doses in aqueous solutions impairs iron absorption [16].

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