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Original article

Appraisal of Anti-anemic potential of food and synthetic source of iron in women of childbearing age

Muhammad Abdullah[1], *Zaheer Ahmed*[1] *Shahid Mahmood*[2], *Hajra Ahmad*[1]

[1] Allama Iqbal Open University (AIOU), Islamabad, Pakistan, Department of Environmental Design Health & Nutritional Sciences

[2] University of Sargodha, Sargodha, Pakistan, Institute of Food Science and Nutrition (IFSN).

Summary: *Background:* Iron deficiency anemia (IDA) is a worldwide public health problem that is most common among females of reproductive age residing in developing countries. Since poverty is the main cause of poor nutritional status, dietary interventions play an imperative role to alleviate such nutritional deficiencies as IDA. *Aim*: Main objective of present research work was to evaluate the anti-anemic efficacy of natural and synthetic iron sources among women of reproductive age. *Methods*: In the current study, various treatments composed of natural and synthetic iron sources were urbanized and analysed for proximates, minerals, calorific values and sensory characteristics fortnightly for two months and their effectiveness was checked among anemic non-pregnant women for 90 days. *Results:* Blood samples of 862 non-pregnant women were collected to investigate their hemoglobin (Hb) status and 36.43 % subjects were scrutinized as anemic (Hb < 11 g/dL). Moreover, blood of these anemic women was investigated further to establish IDA among victims. Hence, 65.92 % non-pregnant females were recognized as IDA prey. At last, 200 volunteers were randomly divided into 4 treatment groups. *Conclusions:* All treatments except placebo (T_0) delivered 9.0 mg of Fe (50 % RDA) among which T_2 showed significant variations (*P-value* <0.05) in dietary intakes and hematological indices during the study span of 90 days. Safety of intervention was revealed by liver function tests (LFTs) and renal function tests (RFTs) of volunteers. The present research work showed that natural iron sources are easily available, cheaper and healthier foods that contained highly bio-available Fe in comparison to ferrous sulphate in alleviating IDA among non-pregnant women.

Key words: Food iron sources, medicinal iron, non-pregnant women, IDA

Introduction

Anemia is a pathological condition which is established when hemoglobin (Hb) level of non pregnant women is 8 to12 g/dL. According to WHO, anemia occurs when hemoglobin level is less than 13 g/dL in man and 12 g/dL in women. It is a significant global health problem that affects individuals of all age and economic classes. Particularly, it predominates among females of reproductive age because conception, delivery, menstruation and breastfeeding increase the iron requirements of body (1). Hb deficiency and IDA prevalence in non-pregnant ladies was 49.6 and 19.9 %, respectively in Pakistan (2). Persons suffering from iron deficiency anemia have inadequate iron intake, mal-absorption, increased physical trouncing during hematolysis, intestinal hemorrhage and severe inflammatory problem (3). Multigravida is the leading cause of anemia in women and their young one. Iron is an inorganic substance that is necessary for growing animals and plants. Dearth levels of this mineral may check metabolic activities in living entities. Iron bounds to its carrier Transferrin (Tf) which is involved in the uptake of iron through its surface receptors (TfR). Labile cellular iron can deposit in red blood cells (RBC) when iron is in excess to bind with

Transferrin receptors during artificial blood supply (4). Excessive iron may lead to several undesirable events such as poor absorption by gastrointestinal tract (5). Various micronutrients and phytochemicals of natural food sources influence iron absorption acting as iron inhibitors (polyphenols and tannins) or enhancers (vitamin C and αtocopherols). Vitamin C and other organic acids should be regularly included in diet to enhance the absorption of iron when plant sources are consumed for iron intake. Blackstrap molasses has high acceptability for supplementation of various products. It is a rich source of Fe, K and Ca. It has high iron content i.e. 250 mg/ Kg (6). Dates are in worldwide consumption especially in Middle East and widely consumed in the advanced nations due to their multiple benefits. These are rich in Potassium, Calcium, Magnesium and Phosphorus. Dates are also a good source of iron and 0.90 mg/100 g iron is present in it. Apricot has a prime role in promotion of human health. It is a rich source of iron, potassium (K) and αcarotene. It contained 400 mg/Kg iron content (7). Rape seed is a good source of iron too which contributes 100 ug/g iron while 3.07 mg dried ferrous sulphate contain 1 mg iron (8). Pakistan ranks fifth in case of sugarcane production and every year 2 million tons molasses is produced. Blackstrap molasses contains fumaric acid which chelates iron thereby improves its absorption and more than 95 % iron in blackstrap molasses is available and it is also devoid of toxic heavy metals. It is cheap, easily available and is a useful source of iron and is waste material of sugar industry. Keeping in view above mentioned facts about Blackstrap molasses and other related sources, this interventional study was designed to combat IDA in non-pregnant women through various natural foods and synthetic source of iron to discourse their impacts.

Material and methods

Product development and evaluation of treatments

Different treatments comprising of natural and synthetic Fe source were developed as given in Table 1 and evaluated fortnightly for proximates, minerals, calorific values and sensory attributes for 2 months and their efficacy was tested for 3 months among non-pregnant women suffering from IDA.

Appraisal study of iron treatments

Ethical review of research project

The study was approved by the Departmental Review Committee for Ethics (DRCE), Institute of Home and Food Sciences (IHFS), GC University, Faisalabad bearing letter no. GCUF/IHFS/12-001 dated 23-01-2012 (9).

Inclusion and exclusion criteria

The non-pregnant females (15-49 years) having Hb < 11.0 g/dL, s. Ferritin (serum Ferritin) < 12 ug/L (Milman, 2011), s. Fe (serum Iron) < 60 ug/dL, TIBC (Total Iron Binding Capacity) > 350 mg/dL, TS (Transferrin Saturation) < 15 % and mean corpuscular volume (MCV) < 80 fL were included while women with hepatic and renal diseases, hemorrhoids and/or excessive menstrual bleeding were excluded from the study. The deworming of selected volunteers was done by Praziquantel @ 40 mg/Kg body weight (10) following a fortnight booster dose prior to baseline.

Table 1. Detail of various treatments of iron

Treatments	Composition	Dose	Fe Content (mg)
T_0	250 mg Lactose as filler in capsule (control)	2 Capsules	00.0
T_1	23.63 g Blackstrap molasses (viscous liquid; net wt: 24 g in sachets)	2 Sachets	09.0
T_2	17.10 g Blackstrap molasses + 3.0 g dried Dates + 3.0 g dried Apricots + 3.0 g Rape seeds (semi solid paste; net wt: 26.50 g in sachets)	2 Sachets	09.0
T_3	13.84 mg Ferrous sulphate + 236.16 mg Lactose in capsule	2 Capsules	09.0

*Non-pregnant women were provided treatments to meet 50 % Fe RDA

Study site and permission from concerned authorities

Written informed consent was taken from women who want to be part of the research project. Twenty Population Welfare Centers (PWCs) were study sites which were selected after approval of District Population Welfare Officer, Department of Population Welfare, Faisalabad, Government of Punjab-Pakistan (11).

Study design, sampling technique and sample size

The study design for research project was randomized controlled clinical trial. The selection of human subjects was carried out in accordance to two stage sampling. Adopting the above method, the required sample size was 2585 women. By considering economy and convenience, 862 (1/3rd of required sample size) non-pregnant women (12) randomly selected and screened for Hb level through B-Hemoglobin Photometer (Model: K061047 HEMOCUE AB, Angelholm, Sweden) among which 36.43 % were found to be anemic (Hb < 11 g/dL). At last, 200 volunteers were randomly divided into 4 treatment groups of 50 volunteers each (Figure 1).

Biochemical investigations

The blood samples of the selected volunteers were collected in two pre-coded tubes, wrapped in Aluminum foil, kept in an ice box and shifted to Food and Nutrition Lab. for further analysis. The blood of tube-I was analyzed for ABO blood grouping and Complete Blood Count (CBC) like Red blood cells (RBC), Hemoglobin (Hb), Hematocrit (Hct), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC) and Mean corpuscular volume (MCV) that were analyzed through fully Automatic Blood Analyzer, Nihon Kohden, Japan. Serum was extracted (13) from blood of tube-II by centrifuge machine (Model: 800, Centrifugal Machine, China). Serum Fe (s. Fe) and total iron binding capacity (TIBC) were estimated by Colorimetric method through Microlab-300, Merck Germany while serum ferritin (s. Ferritin) was analyzed by Immunoassay technique through Access 2 (Model: 510-K, Beckman Coulter, Inc. USA) and transferrin saturation (TS) was determined by the respective formula.

Demographics and anthropometrics

Anthropometric measurements, hematological and biochemical investigations were the research instruments for the said project (14). Demographics such as family income and anthropometric measurements of the selected participants were recorded (15).

Assessment of dietary intakes

Food Frequency Questionaire (FFQ) and Food Diary were used to assess dietary intakes at baseline and during the intervention, respectively. Clinical signs and symptoms and vital signs were also recorded (16).

Distribution of treatments

The non-pregnant women were randomly and separately assorted in to T_0, T_1, T_2 and T_3 groups. Each volunteer had been given daily two capsules/sachets of assigned treatment to meet their 50 % RDA (9.0 mg) of Fe. Every volunteer visited PWC on weekly basis and collected her respective treatment in a packet containing 7 doses and submitted Food Diary and empty packets. At completion of study, all volunteers had visited PWCs for their anthropometrics, energetics and vital signs recording. Blood samples were recollected and analyzed as described earlier in detail.

Statistical Analysis

Data collected was analyzed by using the statistical software SPSS-20. Descriptive statistics was applied to corroborate data distribution and frequency. Collected data was further analyzed through one way analysis of variance (ANOVA) technique and LSD test was used to find out significance ($p \leq 5$ %) between groups. The results obtained after the statistical analysis for various iron treatments of intervention were interpreted logically and accordingly conclusions were drained.

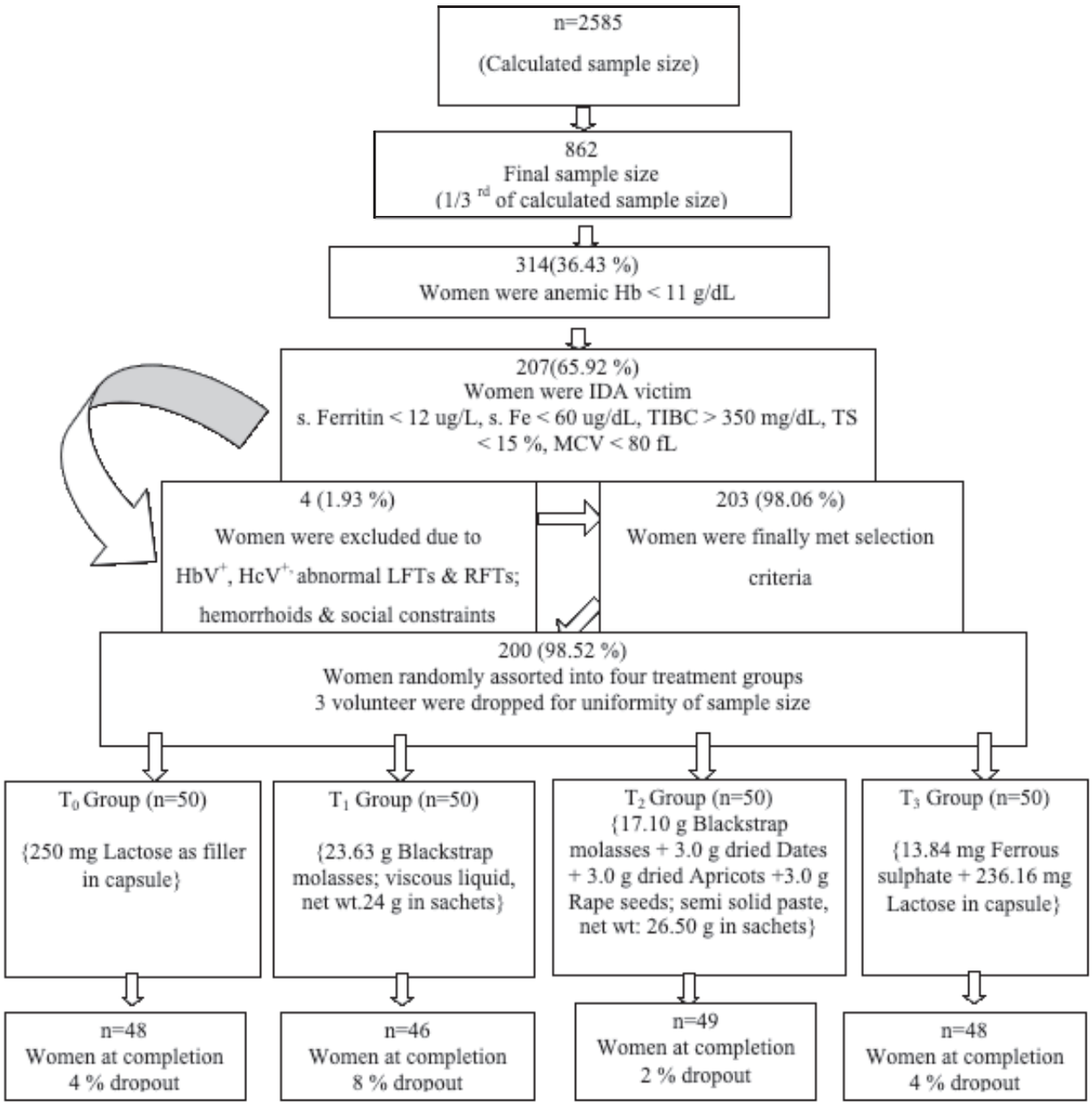

Figure 1. Flow diagram of non-pregnant volunteers' screening and assortment

Results and discussions

Demographics of volunteer

Unmarried non-pregnant subjects that belonged to low SES were 56% which was in accordance to a study in which 61.5 % women had similar low income or low socioeconomic background who had never participated in any nutrient supplementation programme (17).

Dietary Intakes

The significant variations in dietary intakes were observed in non-pregnant women during the study span of 90 days (Table 2). These volunteers were taking

Table 2. Dietary intake (Intake of water and no. of food group servings of volunteers)

Food Group n=184		Days 0 Mean	Days 90 Mean	P. value
Water intake (L/Day)		1.387±0.026^{B}	1.532±0.027^{A}	0.005
a	No of servings	7.60±0.011^{B}	7.70±0.028^{A}	0.003
b	No of servings	0.73±0.001^{B}	0.80±0.011^{A}	0.004
c	No of servings	1.72±0.001^{B}	1.75±0.008^{A}	0.002
d	No of servings	1.63±0.001^{B}	1.68±0.007^{A}	0.003
e	No of servings	1.57±0.001^{B}	1.61±0.007^{A}	0.004
f	No of servings	3.19±0.008^{B}	3.30±0.017^{A}	0.002
g	No of servings	1.68±0.007^{A}	1.63±0.001^{B}	0.001

a: Bread, Cereal, Rice and Pasta Group; b: Fruit Group; c: Vegetable Group; d: Meat, Fish, Poultry, Beans, Eggs and Nuts Group; e: Milk, Yogurt and Cheese Group; f: Fats, Oils and Sweets Group; g: Junk Food Group; Data is expressed as mean values (SEM); SEM: Standard error of mean; *P* value of all food groups is < 0.05 at 90 days of study.

appropriate serving from bread, cereal, rice and pasta group while less serving from most of the remaining group in comparison to the Food Guide Pyramid recommended servings per day. A study conducted on assessment of dietary intake and nutritional status elaborated that intake pattern of most food groups was inadequate which supported the current study results (18). In the current scenario, less than 10 % US college students thought that they were consuming recommended five servings of fruits and vegetables. Some other authors investigated (19) that augment in unhealthy dietary habits and increased consumption of sweetened beverages by young people had an important role in prevalence of under nutrition and insufficient dietary intakes were significantly associated with increased risk of anemia. These findings are further strengthened by other researchers who reported similar interpretations during iron supplementation in anemic subjects (20). A positive correlation was found between Hb levels and intake of cereals, milk and milk products. In this regard a study (21) proved that utilization of milk and milk products reduced the likelihood of anemia.

Anthropometrics

A non significant variation in anthropometric measurements was explored during the study span of 90 days. In a study of 245 female participants, double fortified salt was intervened for a period of 9 months. At the end of study, fortified group had significant improvement in anemia indices (22) but their anthropometric measurements (height, weight and BMI) and energy level showed non significant variations. Eighty two anemic women were randomly divided in to two groups out of which first group was provided Moringa powder while the second group administered on Ferrous Sulphate along with 0.5 mg vitamin C (23). At the end of study, body mass index (BMI) changed from 23.2 to 23.0 Kg/m^2 in the Moringa supplemented group and 22.8 to 22.3 Kg/m^2 in the control group (Ferrous Sulphate) that is quite comparabe to the findings of this study.

Indices of IDA

Although many reported studies revealed iron supplementation in larger doses for management of IDA yet occasionally they provoke GIT discomfort. Some authors quoted iron interventional studies that reduce IDA at small doses. Globally, some studies of natural iron sources for the regeneration of Hb level are available. Therefore, focus should be rigorous on food based approaches to cope with such challenge so as to improve the public health standard on sustainable basis. In a poor country like Pakistan where poverty and micronutrient deficiency is a major problem of common people, present study was done so as to restrain easily available, economical and rich source of natural iron.

In the current study, a highly significant increase in almost all anemia indices i.e. RBC count (5.51 %), Hb (23.83 %), Hct (7.62 %), MCV (0.91 %), MCH (2.66 %), MCHC (2.15 %), s. Fe (4.23 %), s. Ferritin (23.83 %) and TS (33.53 %) while a highly significant decrease in TIBC (4.93 %) was noted among volunteers by the ingestion of treatment T_2 after three months (Table 3 and Figures 2a-2d). Increase in Hb level was greater by T_2 as compared to other treatments which might be due to fact that it was a

Table 3. Biochemical indices of IDA of non pregnant volunteers

Biomarkers	Treatments	Days		P. value
		0	90	
		Mean	Mean	
Total red blood cells (M/uL)	T_0	4.99±0.007^{c}	4.89±0.008^{f}	0.009
	T_1	4.97±0.010^{d}	5.17±0.009^{b}	0.004
	T_2	4.97±0.009^{d}	5.26±0.008^{a}	0.000
	T_3	4.94±0.008^{e}	5.15±0.008^{b}	0.007
Hemoglobin (g/dL)	T_0	9.67±0.149b	9.37±0.149^{bc}	0.006
	T_1	9.08±0.109c	11.74±0.045^{a}	0.003
	T_2	9.08±0.148^{c}	11.92±0.073^{a}	0.001
	T_3	9.51±0.064^{b}	11.71±0.077^{a}	0.005
Mean corpuscular volume (fL)	T_0	77.07±0.113^{c}	76.95±0.104^{c}	0.007
	T_1	77.03±0.118^{c}	77.50±0.117^{b}	0.005
	T_2	77.12±0.088^{c}	77.83±0.092^{a}	0.000
	T_3	77.16±0.060^{c}	77.55±0.056^{b}	0.006
Serum iron (ug/dL)	T_0	47.66±0.200^{b}	46.93±0.174^{c}	0.006
	T_1	47.93±0.204^{b}	48.82±0.193^{a}	0.005
	T_2	46.92±0.148^{c}	48.99±0.165^{a}	0.002
	T_3	46.91±0.178^{c}	47.90±0.198^{b}	0.005
Serum total iron binding capacity (ug/dL)	T_0	403.10±1.120^{c}	416.49±0.968^{a}	0.009
	T_1	407.34±0.809^{b}	389.34±0.808^{e}	0.004
	T_2	405.46±0.771^{bc}	385.46±1.404^{f}	0.002
	T_3	407.69±0.740^{b}	392.68±1.026^{d}	0.004
Serum ferritin (ng/mL)	T_0	9.81±0.297^{d}	7.01±0.266^{e}	0.006
	T_1	9.55±0.149^{d}	11.55±0.149^{b}	0.004
	T_2	10.61±0.242^{c}	13.93±0.248^{a}	0.000
	T_3	9.75±0.246^{d}	11.20±0.241^{bc}	0.003
Serum transferrin saturation (%)	T_0	11.98±0.176^{d}	8.83±0.171^{e}	0.005
	T_1	12.01±0.168^{d}	16.90±0.159^{b}	0.003
	T_2	12.23±0.176^{d}	18.40±0.182^{a}	0.000
	T_3	12.32±0.159^{d}	16.00±0.169^{c}	0.003

Day 0: At baseline before intervention (Pre intervention); Day 90: After intervention of 90 days (Post intervention).

combination of food ingredients like blackstrap molasses, apricots, dates and rape seeds that contained iron in appreciable quantity. According to USDA Branded Food Products Database (6); Molasses (45238944), Dates (45211302), dried Apricots (45029551) and raw Turnips (11564) contained 24.00, 0.90, 2.70 and 0.30 mg Fe per 100 g, respectively. A study regarding the intervention of various natural iron sources especially blackstrap molasses (as a major natural iron food) proved that this by product effectively improved anemia indices. In that study, a highly significant increase in Hb (17.44%), s. Fe (2.45%), s. ferritin (5.61%) and TS (10.20%) was noted while TIBC was decreased by 4.79 % which also verified the results of current study (24). Some researchers worked to gauge the impact of different plant sources and animal liver in anemic

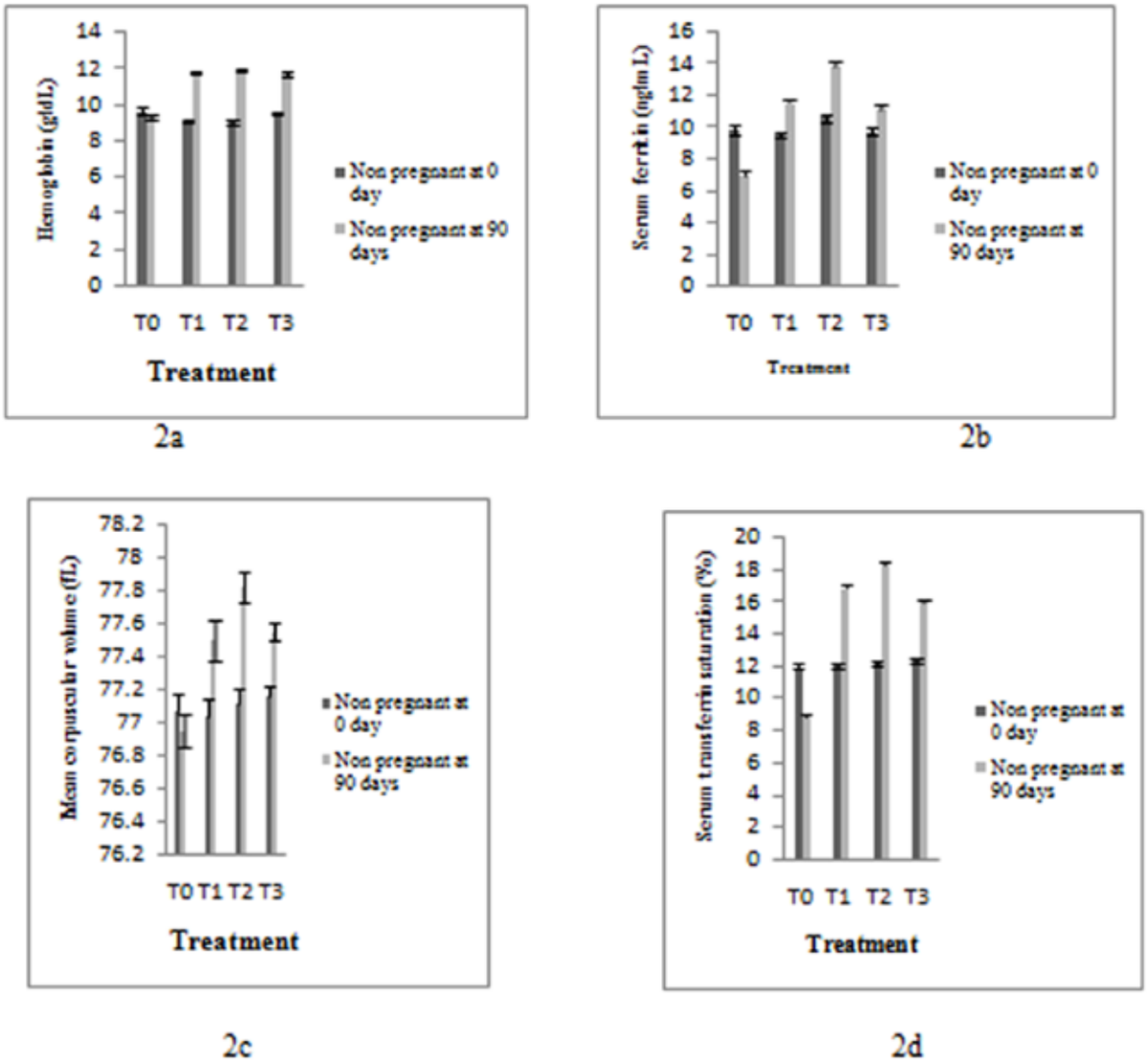

Figure 2. Column graphs showing mean variation in hematological variables of hemoglobin (2a), serum ferritin (2b), mean corpuscular volume (2c) and serum transferrin saturation (2d) of non pregnant women at start of intervention (0 day) and after the end of study (90 days) by ingestion of various treatments of iron such as T_0 (control),T_1, T2 and T_3. All treatments had $P < 0.05$ at post intervention phase in comparison to the pre intervention phase. *P*-value is taken from paired t-test.

Egyptian females of childbearing age (25). After the intervention of stated period, Hb, s. Fe and s. Ferritin amplified from 9.90±0.46 to 11.40±0.10, 35.67±2.40 to 53.00±2.89 and 15.67±0.88 to 30.33±1.45 in black-strap molasses and 9.53±0.19 to 11.60±0.21 g/dL, 29.00±1.86 to 50.00±2.00 ug/dL and 15.00±0.33 to 31.00±2.08 ug/L in black dates, respectively. It was observed that aubergine, black dates and buffalo liver positively enhanced Hb, s. Fe and s. Ferritin levels in comparison to watercress and molasses. In a study, diet of reproductive age women (15-49 years) was supplemented with Hibiscus feast @ 1.9 kg per day (1.71 mg iron per 100 g feast) and supplementation frequency was kept thrice weekly for 3 months. Significant improvement in iron status of volunteers was recorded after the completion of intervention (26).

A similar lunch programme (n=400) comprising of local foods and soybean was launched five times a week for eight months. After the end of stated programme, significant positive effects on hemoglobin and red blood cells of participants were noted (27). A study on coffee cultivation workers was conducted in three phases. An increase of 21 % in blood Hb levels along with a considerable positive rise of s. Fe and s. ferritin was recorded in the studied subjects. However, TIBC decreased significantly showing an improvement in the hematological variable too. In another study, iron fortified lactoferin bovine colostrums was administered to 68 anemic women for one month and significant positive effects on patients Hb, s. Fe and TIBC were examined after the study (28). LFTs and RFTs of non-pregnant volunteers varied non-significantly by all treatments and study duration. In this regard, linear variations were reported during a study on pregnant volunteers in whom intervention of blackstrap molasses was done (24).

Conclusion

Except placebo (T_0), all treatments contained 50 % RDA of Fe. Variations were non significant in case of anthropometrics and energetics while these were significant regarding dietary intakes. Moreover, significant improvement in hematological indices of volunteers was depicted by treatment T_2. LFTs and RFTs of volunteers revealed the safety of the dietary intervention. The current study showed that plant foods like Blackstrap molasses, dried Apricots, dried Dates and Rape seeds are economical and highly bio-available Fe iron sources as compared to Ferrous sulphate in skirmishing IDA.

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Address Correspondence:
Dr. Zaheer Ahmed, Associate. Professor,
Department of Environmental Design Health & Nutritional Sciences
Allama Iqbal Open University Islamabad
Pakistan
Tel. 0092-519057265
Fax: 0092-519250063
E-mail: zaheer_863@yahoo.com