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# Evaluation of anti anemic prospective of natural iron sources in lactating women- an ignored important segment of Pakistani population

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#### Abstract

Almost entire segment of expecting and breastfeeding mothers in Pakistan are severely affected by iron deficiency anemia (IDA) with predictable prevalence of 50% and as severe public health problem in lactating mothers, has worldwide pervasiveness. Commonly used synthetic iron sources provoke complications. Thus, food sources are preferred due to high bioavailability and sustainability. The present study was done on various natural and synthetic iron sources evaluated for proximate composition, mineral matter, sensory attributes and storage stability. The treatments were fed for 90 days and their efficacy was tested among anemic lactating women. The blood samples of 862 women were taken to examine their hemoglobin level and pertinent biomarkers. Hence, 37.00% volunteers were found to be anemic (Hb < 11 g/dL) and 67.71% women showed IDA problem. Finally, 200 volunteers were strewn arbitrarily into 4 groups. All treatments provided 50% RDA of iron. Dietary intakes and biochemical investigations of  $T_2$  group showed momentous improvement (P < 0.05) while linear variations were recorded in anthropometrics, liver function tests (LFTs) and renal function tests (RFTs). Study revealed that food iron sources are easily accessible, cheaper, safe and has more bioavailable iron in skirmishing IDA among lactating women on sustained basis.

Keywords: food iron; synthetic source; lactating women; IDA.

**Practical Applications:** The investigated natural iron sources i.e. molasses and dried fruit may be effectively used in the food processing industry for the production of fortified food products so as to address or minimize or prevent nutrient deficiencies in the deficient population.

#### 1 Introduction

Anemia is a condition in which blood hemoglobin level is reduced from normal concentration (Hb < 11 g/dL in lactating women) and it hinders the supply of oxygen to blood which badly distress mother and child health, newborn development, working competence, astuteness and earning power of employment span (Sathyamala, 2017). These effects among community lead to significant economic setback due to inevitable GDP and disease management expenses. Anemia affects one third of adult population and more than 2 billion inhabitants on the globe. Predominantly it occurs among females of childbearing age because menstrual cycle, pregnancy, child birth and lactation increase iron requirements of body (Kassebaum et al., 2010). Unsurprisingly, entire segment of expecting and breastfeeding mothers in Pakistan are seriously affected by IDA and probable incidence of this malady among women of childbearing age is fifty percent. Due to this reason, maternal and child mortality rate is high in Pakistan. Anemia prevalence among Indian lactating mothers is reported to be 63% (Siddiqui et al., 2017). When prevalence of anemia is  $\geq 40\%$ , public health problem is severe in nature (Mhanna et al., 2016). Symptoms of IDA are lassitude, tiredness, ringing in ears, paleness, reduced immunity, impaired growth, watery conjunctivae, taste disorder, spoon nails, ice craving and glossitis. Decrease in Hb concentration during pregnancy is about 10%. Postpartum blood volume decreases according to blood loss during child birth and, Hb levels also decreases straight away after the delivery due to fluids reorganization. Iron is necessary for some fundamental body functions such as oxygen transportation to cells and Hb regeneration by erythropoietin mechanism. Synthesis of Hb and red blood cells will not occur if iron supply is inadequate (Milman, 2011).

Iron binds to carrier transferrin which uptakes iron through receptors. Excessive iron unable to attach with receptors is labile cellular iron that may abandon in red blood cells particularly blood administration during chronic anemia (Wessling-Resnick, 2017). Iron deposited in excess may not absorb efficiently in the gastrointestinal tract (GIT). Various indispensable nutrients of food origin that influence iron absorption (Cepeda-Lopez et al., 2015) are termed as dietary inhibitors (polyphenols and tannins) and enhancers (vitamin C and α-tocopherols). Blackstrap molasses is a treasure of minerals that gives more iron than red meat, builds iron stores, contains fewer calories and its two tablespoonfuls provide 13.3% RDA of iron. It is very useful during menstruation, breastfeeding, pregnancy and also for growing children (Jain & Venkatasubramanian, 2017). Dates are rich source of minerals which express their vital role in hemoglobin and bone make up (Saryono & Rahmawati, 2016). Apricot is a rich source of iron, potassium, beta-carotene and is useful for

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bone, teeth and Hb synthesis (Yilmaz et al., 2012). Blackstrap molasses, dates and apricot contain 25, 0.58 and 40 mg / 100 g bioavailable iron, respectively. Rape seed is also a rich source of iron (10 mg/100 g) while 3.07 mg elemental ferrous sulphate powder contains 1 mg iron (US Department of Agriculture, 2018). In Pakistan, 2 million tons molasses is produced every year which is the main by-product of sugarcane industry and its recovery is about 5% of the sugarcane plant. The current study focused the important ignored segment of lactating women in Pakistan while considering cheap, easily available, abundant and waste material of sugar industry such as blackstrap molasses (already being used as animal feed in the country). Therefore, the prime objective of the study was to investigate the nutritional profile of various natural iron sources. Moreover, impact of natural iron sources and Ferrous Sulphate on IDA in lactating mothers was compared and correlation between dietary intakes and IDA was also established.

### 2 Materials and methods

Four treatments of food and synthetic sources of iron were formulated (Table 1) and qualitatively analyzed for proximates, mineral composition, calorific content and sensory attributes fortnightly for 60 days and their impact was evaluated after 90 days among lactating women who were screened to be IDA patient.

## 2.1 Efficacy studies of iron treatments

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Departmental Review Committee for Ethics (DRCE), Institute of Home and Food Sciences (IHFS), GC University, Faisalabad vide letter no. GCUF/IHFS/12-001 dated 22-01-2016. The study was also approved and registered in the BASAR committee meeting of Allama Iqbal Open University (AIOU), Islamabad held on 04-02-2016 under the chairmanship of Vice Chancellor of AIOU vide letter no. AIOU-VC/HHS/30-55 dated 04-02-2016 (Zoccatelli et al., 2018).

The lactating females (15-49 years) with Hb < 11.0 g/dL (Sathyamala, 2017), serum ferritin (s. Ferritin) < 12 ug/L (Milman, 2011), serum iron (s. Fe) < 60 ug/dL, total iron binding capacity (TIBC) > 350 mg/dL, transferrin saturation (TS) < 15% (Manavifar et al., 2014) and mean corpuscular volume (MCV) < 80 fL (Anand et al., 2018) were selected as participant of the study while women having liver and kidney problems, hemorrhoids and/ or acute menstrual bleed were disqualified for the study. The selected volunteers were dewormed by Praziquantel administered as 40 mg per kg body weight (Inobaya et al., 2018)

followed by a fortnight booster dose. Prior written consent of the female to be a participant for the current research study was taken. Study site selected after approval of concerned authority consisted of 20 Population Welfare Centers (PWCs), Faisalabad, Government of Punjab-Pakistan. 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 (Nath, 2015). By considering economy and convenience, 862 (1/3rd of required sample size) lactating women randomly selected and screened for Hb level through B-Hemoglobin Photometer (Model: K061047 HEMOCUE AB, Angelholm, Sweden); 37.00% women were found to be anemic (Hb < 11 g/dL). At last, 200 volunteers were allocated randomly into 4 treatment groups of 50 volunteers each (Figure 1). Their blood was 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 CBC like red blood cells (RBC), Hb, hematocrit (Hct), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and MCV that were analyzed through fully Automatic Blood Analyzer, Nihon Kohden, Japan. Serum was extracted from blood of tube-II by centrifuge machine (Model: 800, Centrifugal Machine, China). Serum Fe and TIBC were estimated by Colorimetric method through Microlab-300, Merck Germany while s. Ferritin was analyzed by Immunoassay technique through Access 2 (Model: 510-K, Beckman Coulter, Inc. USA) and TS was determined by the standard formula. The study design for research project was randomized controlled clinical trial (Spieth et al., 2016). Anthropometric measurements, hematological and biochemical investigations were the research instruments for said project (Sharami et al., 2017). Demographics such as volunteers' family income etc. and anthropometrics of the selected subjects were recorded (Anand et al., 2018). Food frequency questionnaire (FFQ) and Food Diary were used to assess dietary intakes at baseline and during the intervention. Clinical signs and symptoms and vital signs such as body temperature (°F), blood pressure (mmHg) and pulse rate (beats/minute) of all the selected volunteers were also recorded throughout the study (Herranz-Lopez et al., 2019). Lactating volunteers were randomly allocated in to T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> groups. Each volunteer was daily fed one capsule / sachet of allocated treatment so as to congregate their 50% RDA (4.5 mg) of Fe. Every volunteer visited PWC once a week and collected a packet of her individual treatment containing 7 doses and brought back Food Diary and empty packets. At the end of study, all volunteers paid a visit to PWCs for the assessment of anthropometrics, energetic and vital signs recording. Blood samples were recollected and analyzed as described earlier in detail.

Table 1. Detail of various treatments of iron.

Treatments	Composition	Dose	Fe Content (mg)
$T_{o}$	250 mg Lactose as filler in capsule (control)	1 Capsule	00.0
$T_{_1}$	23.63 g Blackstrap molasses (viscous liquid; net wt: 24 g in sachets)	1 Sachet	04.5
$T_2$	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)	1 Sachet	04.5
T <sub>3</sub>	13.84 mg Ferrous sulphate + 236.16 mg Lactose in capsule	1 Capsule	04.5

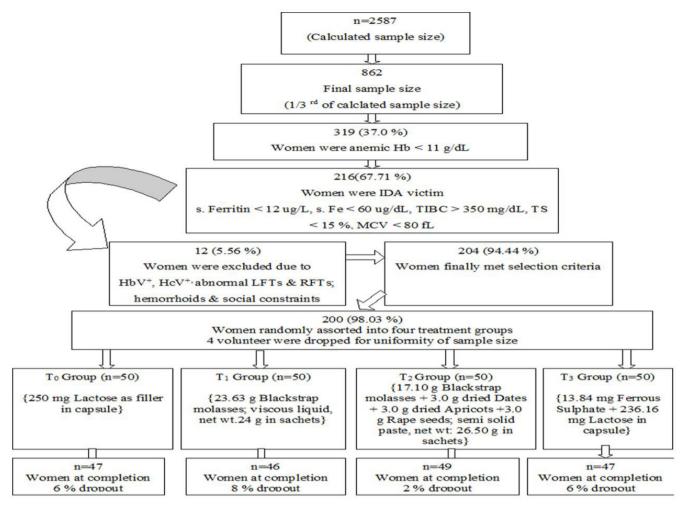


Figure 1. Flow diagram of lactating volunteer' screening and assortment.

## 2.2 Statistical analysis

Data collected during interventional study was analyzed with the help of statistical software SPSS-20. Data distribution and frequency was examined by applying the descriptive statistics. One way analysis of variance (ANOVA) technique was utilized to further analyze the collected data set and significance (p  $\leq$  5%) between groups was established through LSD test. Paired t-test was used to find out the correlation between variables. The results of the statistical analysis so obtained for various treatments of iron intervention were interpreted on logical grounds and consequently conclusions were drawn.

#### 3 Results and discussion

## 3.1 Demographics of volunteers

Demographic variable of mean age (years) and married years of the lactating women were 29.37 and 7.93, respectively. Number of children and family members (no.) were found to be 2.82 and 8.54, respectively. History of abortion, miscarriage and premature births was 2.17, 0 and 2.17%, respectively. Joint family system was 96.20% and nuclear family system was found in 3.80% subjects. In this group, 70.11, 16.85 and 13.04%

mothers reported breastfeeding, breastfeeding plus bottle feeding and no breastfeeding (bottle feeding), respectively. Regarding lactation period; 42.39, 9.78 and 9.24% mothers explained < 1 year breastfeeding, breastfeeding plus bottle feeding and no breastfeeding (bottle feeding), respectively. Majority of the mothers (90.22%) had sedentary level of physical activity while family income was less than Rs. 39000/- in 7.61% and less than Rs.20000/- among 92.39% women (Table 2). In a study of 327 Ethiopian lactating females (Egata, 2018), demographic data and anemia associated risk factors were collected on the predesigned questionnaires. Majority of illiterate mothers with poor socioeconomic status, multiple gravida and low dietary diversity score (intake from less food groups than standard) from the age group 25-34 years were anemic (28.7%) and history of abortion was acknowledged in 9% of these volunteers. Low diet diversity score leads to inadequacy of vitamins and minerals along with compromised iron bioavailability resulting in the higher anemia events. In the present study, low abortion history was found that might be because the study site was urban area with better health facilities. A survey conducted in Sub-Sahara Africa (Gebremedhin, 2019) showed exclusive breastfeeding and bottle feeding at first (12-15 months) and second year of

Table 2. Demographics of volunteers.

Demographics	Lactating Women (n = 184)		
	f	%	
In the family marriage	135	73.37	
Out of the family marriage	049	26.63	
Abortion	004	02.17	
Never Abortion	180	97.83	
Premature Birth	004	02.17	
Never Premature Birth	180	97.83	
Family system (Joint)	177	96.20	
Family system (Nuclear)	007	03.80	
Breastfeeding (Less than 1 year)	78	42.39	
Breastfeeding (Less than 2 years)	51	27.72	
Breast plus Bottle feeding (Less than 1 year)	18	09.78	
Breast plus Bottle feeding (Less than 2 years)	13	07.07	
Bottle Feeding (Less than 1 year)	17	09.24	
Bottle Feeding (Less than 2 years)	7	03.80	
Family income (Less than Rs.20000)	170	92.39	
Family income (Rs. 21000-39000)	014	07.61	
Physical activity level (Sedentary)	166	90.22	
Physical activity level (Slightly Active)	018	09.78	

n= total no. of volunteers; f= frequency.

study in the participants as 89, 16.6, 53 and 11.7%, respectively. In the study of Africa, percentage of breastfed and bottle-fed babies was comparable to results of our study.

#### 3.2 Anthropometric measurements

After intervention of 90 days, all the treatment groups regarding anthropometrics showed non significant variations as shown in Table 3. Eighty two lactating anemic women of the age group 26.7±6.5 years took either Moringa powder or Ferrous Sulphate (Idohou-Dossou et al., 2011). A non significant variation in weight (58.9 - 58.1 Kg) and body mass index (23.2 to 23.0 Kg/m<sup>2</sup>) of Moringa supplemented group was noted that is in accordance to present study. A randomized controlled clinical experiment was carried out to gauge the effect of dietary supplement on anthropometric measurements in lactating mothers (Kindra et al., 2011). It was explored that the dietary supplement had no significant effect on anthropometrics of breastfeeding mothers. In a study, reproductive age women (15-49 years) daily received 1.9 kg supplement of Hibiscus feast containing 1.71 mg iron / 100 g feast (Kubuga et al., 2019). No significant variations were recorded in anthropometric measurements of volunteers that verify the findings of current study. Some authors (Kundu et al., 2013) investigated that BSA of mild anemic and moderate anemic women was 1.32±0.09 and 1.25±0.05 m<sup>2</sup> respectively. Optimum value of BSA in females is 1.8 m<sup>2</sup> that also supports the findings of the current research study in which BSA after 90 days of intervention was 1.52±0.007 m<sup>2</sup>.

## 3.3 Assessment of dietary intakes

A significant positive impact of study was observed among all treatment groups regarding dietary intakes that might be due to nutrition education and dietary counseling (Table 4).

**Table 3**. Anthropometrics (mean values) of volunteers.

	Lactating (n = 184)					
Variables	Da	y 0	Day 90			
	Mean	SEM	Mean	SEM		
Weight (Kg)	57.67 <sup>A</sup>	0.803	57.23 <sup>A</sup>	0.770		
Height (cm)	$155.02^{A}$	0.379	$154.82^{A}$	0.399		
Body mass index (Kg/m²)	$22.95^{A}$	0.169	$22.96^{A}$	0.169		
Body surface area (m²)	1.52 <sup>A</sup>	0.006	1.52 <sup>A</sup>	0.007		

n= total no. of volunteers. Data is expressed as mean values (SEM). SEM= standard error of mean.

**Table 4**. Intake of water and no. of servings (mean values) of volunteers.

	_		Lactating $(n = 184)$				
Food Groups		Day 0		Day 90			
		Mean	SEM	Mean	SEM		
Water intake (L/Day)		1.528 <sup>b</sup>	0.028	1.660a	0.027		
a	No of servings	$7.58^{b}$	0.012	8.51 <sup>a</sup>	0.069		
b	No of servings	$0.72^{b}$	0.001	$0.89^{a}$	0.014		
С	No of servings	$1.72^{b}$	0.001	$1.75^{a}$	0.006		
d	No of servings	$1.62^{b}$	0.001	1.66a	0.006		
e	No of servings	$1.56^{b}$	0.001	$1.58^{a}$	0.005		
f	No of servings	$3.51^{a}$	0.001	$3.52^a$	0.009		
g	No of servings	$1.66^{a}$	0.006	1.62 <sup>b</sup>	0.001		

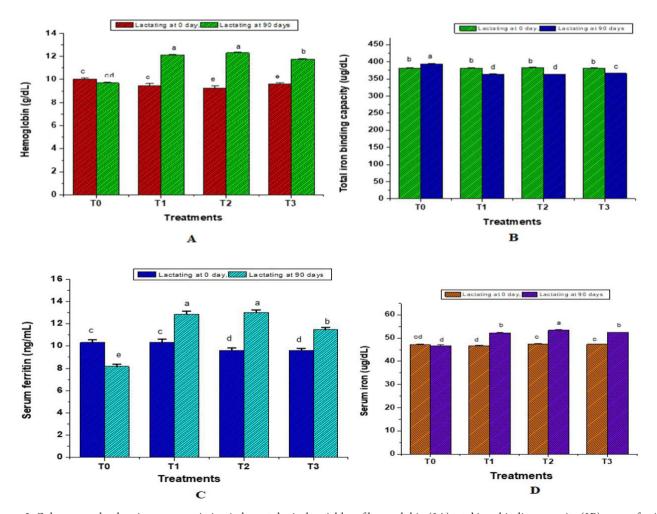
a=Bread, Cereal, Rice and Pasta Group; b= Fruit Group; c= Vegetable Group; d= Meat, Fish, Poultry, Beans, Eggs and Nuts Group; e= Milk, Yogurt and Cheese Group; f= Fats, Oils and Sweets Group; g= Junk Food Group. Data is expressed as mean values (SEM). SEM= standard error of mean. P value of all food groups is < 0.05 at 90 days of study; n= total no. of volunteers.

Intake of most food group servings of all volunteers in present study was less compared to the Food Guide Pyramid recommended servings per day irrespective of the Bread, Cereal, Rice and Pasta Group. A study was conducted on 4,290 women of childbearing age (15 - 49 years) in Ghana (Ghose & Yaya, 2018) so as to assess the prevalence of anemia and its relationship with fruits and vegetable consumption. Women which were not following the WHO recommendations for optimum fruits and vegetable servings, had more incidence of severe anemia that corroborates the results of present study. In pregnant and lactating women from rural area of Niger, assessment of food intakes was done by 24 hours dietary recall method (Wessells et al., 2019). Statistical analysis revealed that daily intake of one additional food serving along with leafy vegetables, vitamin A, yoghurt, pearl millet and beans will minimize the chances of inadequate dietary intakes in the study groups. A trial was conducted to assess food intake and micronutrients status of lactating women in Zambia by estimated average requirement. Half of the women were anemic (50%) and majority of the women (99.9%) had inadequate intakes of all micronutrients assessed by their low dietary diversity score (Kaliwile et al., 2019) which is in agreement to the findings of current study. Nutritional status of Ethiopian lactating mothers using dietary diversity score showed that dietary intakes of only 31.9% participants contained six food groups while 43.6 and 24.4% of volunteers ingested five and less than four food groups, respectively (Egata, 2018). Food abundantly consumed was cereal (94.8%) accompanied by pulses and vegetables. Participants who consumed less than six food groups were more likely to be anemic that is more alike to the current study.

## 3.4 Biochemical investigations

Treatments, study interval and their interaction depicted a highly significant effect on all biochemical indices of lactating anemia that is evident from Table 5 and Figure 2A-D. Biochemical investigations of lactating volunteers were improved significantly by treatment T<sub>2</sub> in 90 days which is proved by following results; TRBC (6.24%), Hb (32.72%), Hct (9.91%), MCV (3.04%), MCH (5.48%), MCHC (4.86%), s. Fe (12.57%), s. ferritin (35.72%), TS (50.64%) and TIBC (5.19%), respectively. Fe supplements commonly used to control IDA are Ferrous sulphate and Ferrous gluconate. But intake of these supplements is also accountable for various complications like depressed growth, abdominal discomfort and nausea. Molasses derived from sugarcane besides raising the iron status of deficient population also act as antioxidant (Asikin et al., 2013). An author reported (Tandon, 2002) that usually allopathic medicines are used to cure various illness and nutritional diseases while natural food sources are ignored. Hence a shift from pill to natural food sources is a hard task but emphasis should be prioritized to convince the public to take up the natural common food simply as the best remedy for the control of IDA.

On hundred ninety three (n=193) females (Mozaffari-Khosravi et al., 2010) were randomly divided in to iron supplemented or un-supplemented group. Iron supplemented group (ISG) used weekly 150 mg Ferrous sulphate for 4 months while un-supplemented group (IUG) took placebo. The study revealed that this low dose iron effectively improved iron status and there is no need for higher doses of iron that is in close agreement to our study. In a study, rats fed on diet of soybean, pulses, sesame seeds, pumpkin seeds, blackstrap molasses and their mix as iron source, had significant rise in anemia indices such as Hb, Hct, MCV, MCH and MCHC (Yun et al., 2011). A research worker (Jain, 2013) formulated biscuits, handwa, idli and soy chat following food fortification strategy. Group one of twice weekly medicinal iron had significant rise in Hb concentration (p < 0.05). Similarly, food iron supplement was also effective in improving the anemia indices (p < 0.05) which maintained the Hb levels of volunteers for 120 days while medicinal iron did not behave so. Hence food based strategy



**Figure 2**. Column graphs showing mean variation in hematological variables of hemoglobin (2A) total iron binding capacity (2B) serum ferritin (2C) and serum iron (2D) of lactating women at baseline (0 day) and after the end of intervention (90 days) by intake of various treatments of iron such as  $T_0$  (control),  $T_1$ ,  $T_2$  and  $T_3$ . All treatments had P < 0.05 at post intervention phase. P-value is from paired t-test.

**Table 5**. Biochemical indices of IDA of Lactating volunteers.

	_	Days				
Biomarkers of Volunteers ( $n = 184$ )	Treatments	0		90		P value
		Mean	SEM	Mean	SEM	
Total red blood cells (M/uL)	T <sub>o</sub>	4.56°	0.009	4.46f	0.008	0.005
	$T_1$	4.59 e	0.010	4.79°	0.011	0.004
	$T_2$	$4.65^{d}$	0.013	$4.94^a$	0.014	0.001
	$T_3$	$4.66^{\rm d}$	0.013	$4.86^{b}$	0.014	0.005
Hemoglobin (g/dL)	$T_0$	10.03°	0.111	$9.70^{\rm cd}$	0.097	0.004
	$T_{_1}$	$9.47^{\mathrm{de}}$	0.200	12.11 <sup>a</sup>	0.076	0.002
	$T_2$	9.29 <sup>e</sup>	0.158	12.33 <sup>a</sup>	0.069	0.001
	$T_3$	$9.60^{\mathrm{de}}$	0.132	11.76 <sup>b</sup>	0.057	0.005
Mean corpuscular volume (fL)	$T_0$	75.97°	0.084	$74.51^{d}$	0.086	0.007
	$T_{_1}$	75.97°	0.080	$78.00^{a}$	0.080	0.006
	$T_2$	76.05°	0.101	78.36 <sup>a</sup>	0.098	0.002
	$T_3$	75.90°	0.104	77.91 <sup>b</sup>	0.105	0.007
Serum iron (ug/dL)	$T_0$	$47.26^{cd}$	0.161	$46.74^{d}$	0.326	0.005
	$T_1$	$46.71^{d}$	0.211	52.23 <sup>b</sup>	0.156	0.004
	$T_2$	$47.40^{\circ}$	0.285	53.36 <sup>a</sup>	0.254	0.003
	$T_3$	47.37°	0.118	52.49 <sup>b</sup>	0.071	0.006
Serum total iron binding capacity (ug/dL)	$T_0$	381.56 <sup>b</sup>	1.041	394.29 <sup>a</sup>	0.662	0.006
	$T_{_1}$	$382.04^{b}$	0.831	$364.06^{d}$	0.710	0.005
	$T_2^{-}$	383.30 <sup>b</sup>	0.682	$363.39^{d}$	0.692	0.001
	$T_3$	381.85 <sup>b</sup>	0.645	366.63°	1.114	0.003
Serum ferritin (ng/mL)	$T_0$	10.32°	0.249	8.16e	0.231	0.004
	$T_1$	10.35°	0.287	12.85 <sup>a</sup>	0.275	0.001
	$T_2^{-}$	$9.60^{\rm d}$	0.252	13.03 <sup>a</sup>	0.238	0.000
	$T_3$	$9.62^{\rm d}$	0.187	$11.48^{b}$	0.210	0.002
erum transferrin saturation (%)	$T_0$	12.92 <sup>d</sup>	0.172	9.91°	0.168	0.004
	$T_{_1}$	13.07 <sup>d</sup>	0.164	19.15 <sup>b</sup>	0.155	0.003
	$T_2$	13.23 <sup>d</sup>	0.173	19.93ª	0.153	0.001
	$T_3^2$	$13.36^{d}$	0.166	18.14 <sup>c</sup>	0.170	0.003

Day 90= after intervention of 90 days (Post intervention). Day 0= at baseline before intervention (Pre intervention); n= total no. of volunteers, SEM= standard error of mean; p value is from Paired t-test.

for controlling anemia has more potential for Hb sustainability. In another study, 150 non lactating anemic females were given daily 100g iron fortified soy flour cookies up to four months. Therapeutic effects of the fortified cookies showed a positive significant increase in Hb, serum iron and white blood cells count (Sari et al., 2018). Food based approaches on community levels in terms of iron fortification for the control of IDA are (Akhter et al., 2014) becoming more popular. In this regard, fresh buffalo milk was fortified with Ferrous sulphate and fed to Sprague Dawley rats to evaluate its impact on IDA. After the intervention for 8 weeks, Hb (g/dL) of experimental groups depicted significant increase (9.84±0.287 to 14.37±0.325) in comparison to the placebo group (p≤0.05). In a study, 195 women (18–27 years) were fed with iron fortified beans (86 mg iron per kg) and normal beans for an interval of four months in Rwanda (Haas et al., 2016). At the end of study, iron fortified beans (food sources of iron containing supplement) depicted significant and greater rise in Hb (3.8 g/l), ferritin (0.1mg/l) and serum iron (0.5 mg/Kg) than control group (P < 0.05).

Efficacy study of a composite product of amla, jaggery and pumpkin foliage was carried out in iron deficiency anemic female youngsters (Resmi et al., 2017). At the competition of study, Hb and serum iron of participants showed positive improvement of 9.94 to 10.99 g/dl and 77.6 to 99.58 ug/dl, respectively. Another similar study was done on 170 female youngsters (Bhuvaneswari et al., 2017) in order to gauge the usefulness of iron-folic acid and a mixture of honey, dates and amla (local common natural food items). After the study, food mixture group showed significant improvement in anemia indices in comparison to the iron group. The author had view that people should be convinced to consume local food items that has beneficial therapeutic efficacy in order to fulfill nutritional deficiencies. Fifty (n=50) medical college students were randomly allocated to receive a dietary supplement; 5 g each of Jaggery ball and raisins (Sakthibalan et al., 2018). Food supplement was provided daily up to two months. At the end of intervention, a significant increase in Hb and total RBC count of female volunteers was recorded without any adverse reaction as often reported in various intravenous and oral administrations. Hence it may be used as preventive strategy to combat IDA in susceptible inhabitants that is in accordance to current study upshot. Iron supplement was provided to 68 anemic young girls (Sari et al., 2018). It was revealed after the study that combined

therapy of iron supplement and dates showed more significant difference in iron status of adolescents than the control one. In a study, reproductive age women (15-49 years) were daily fed with supplement of Hibiscus feast (Kubuga et al., 2019). Iron status of anemic volunteers showed significant positive effects that also verify the findings of current study.

#### **4 Conclusions**

To the best of our knowledge no such study on lactating women has been conducted in Pakistan from the last two decades where majority of people are poor and are not able to adequately feed their family. Due to this reason, not only the health of lactating mother is badly affected but their young ones are malnourished too. Resultantly, maternal and child mortality rate is high in the country. In this study, treatments of synthetic and food sources of iron equally provided 50% RDA of Fe (4.5 mg) among various lactating groups with the exception of placebo (T<sub>o</sub>). Anthropometrics and energetics of lactating women showed non significant variations while dietary intakes of all groups depicted significant effect of the study interval. Response of biochemical investigations of volunteers by treatment T<sub>2</sub> was significantly better and more persistent as compared to all other treatments. Safety of the dietary interventions was confirmed by linear variations recorded during LFTs and RFTs. It may be concluded from the study findings that food sources of iron such as blackstrap molasses have more bioavailable iron in comparison to synthetic source and may be effectively used in anemic lactating volunteers which is an overlooked important segment of the Pakistani population.

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