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SYNERGISTIC EFFECT OF ASCORBIC ACID AND IRON SUPPLEMENTATION AGAINST POSTPARTUM ANEMIA AND OXIDATIVE STRESS MITIGATION

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Abstract:

Background & Objective: Postpartum Anemia is a multi-factorial pathogenic condition, which is etiologically determined by decreased blood cells production and decline in antioxidant levels which fail to fulfill body requirements and result in severe health related complications such as postnatal iron deficiency, inflammation, defective erythropoiesis, hemolysis and oxidative stress. Vitamin C decreases oxidative stress as well as enhance absorption of iron from non heme sources and ultimately increases maternal iron stores by converting oxidation state of iron from (Fe3+) ferric iron to (Fe2+) ferrous iron. Aim of this study is; To determine the role of oxidative stress in denouement of anemia and combined effect of vitamin C along with iron supplementation during postpartum anemia on maternal iron status and oxidative stress.

Methods: RCT was conducted in THQ hospital Muridke, Pakistan .40 patients were included and data that was collected included Demographics, PBAC scoring, CBC, Serum iron, Serum Ferritin, Serum vitamin C, MetHb%, TAC and FFQ. Participants were then divided into 2 groups that were observed for 8 weeks. The control group received iron supplementation (iron poly maltose 100g) 1 tablet per day and experimental group received both iron and vitamin C supplementation (iron poly maltose 100g along with chewable vitamin C 500mg), 1 tablet of both supplements per day in a single dose for 8 weeks. After 8 weeks post analysis of above mentioned parameters was done.

Results: Hb, HCT, MCV and MCH level of experimental and control group was found significant ($p=0.000 < 0.05$). RBCs count in experimental and control group was not significant (P=O.610 >0.05).Results indicated no significant improvement in RBCs count with any supplement. However, Serum iron level, serum ferritin and serum vitamin c levels of experimental and control group was significant ($p=0.000 < 0.05$). Similarly, MetHb% and TAC level of both groups improved significantly ($p=0.000<0.05$).

Conclusion: Vitamin C and iron considerably shown pronounced improvement in Hb, HCT, MCV, MCH, Serum iron, TAC and Serum Ferritin levels and decreases MetHb% in women with postpartum anemia. But the improvement in RBCs count was not significant. Improved TAC level and decline in MetHb% after treatment indicate that vitamin c improves oxidantal defense within body.

Key words: PPA, Hb, Serum iron Level, Serum Ferritin Level, Serum vitamin C, MetHb%, TAC

INTRODUCTION

Anemia is becoming one of the leading health related issues in post-delivery complications worldwide nowadays which is also termed as postpartum anemia. This condition is described by WHO as Hb levels less than (<11g/dl) at one week after delivery and (<12g/dl) within a year of delivery. Its prevalence increased in developed countries by (22-50%) and in developing countries by (50-80%) [1]. It is listed as one of the four major nutritional diseases in the world .It occurs when intake, excretion and stored iron is not enough to keep up erythropoiesis. Heme protein is most abundant and considered as the best prediction of anemia. [2].Severe anemia affects the wound healing process and causes several breast infections with a bad impact on both child and mother health [3].

If maternal iron stores aren't restored immediately after delivery, negative health consequences of postpartum iron deficiency persist throughout the life, majorly if diet contain low bio available iron and if Inter-pregnancy interval is less than 18 months [4]. (MONITOR) advisory group advice postpartum anemia measurements at three different time periods during the first 6 weeks, 6 months and a year of postpartum [5].Erythropoiesis related iron demand is created by three variables. Oxygenation of tissues, turnover rate of erythrocytes and loss of erythrocytes through hemorrhage [2].

Oxidation in cellular components damage normal endogenous cellular antioxidant system [6], leads body towards oxidative stress, a condition characterized as a potential mechanism in the pathogenesis of IDA [7]. If the mechanism through which oxygen reactive species are normally removed enzymatically and non-enzymatically deranged or distract oxidation dominates results in oxidative stress [6].Red blood cells are the primary site of oxidative stress as their function is the transportation of oxygen. Destruction of them in oxidative stress lead toward anemic condition [8].Free radicals can also be produced by iron during Fenton reaction but naturally scavenging mechanisms are present within body to regulate the absorbed amount of iron [9].

Labialise iron and O_2- are the primary reactive species to cause oxidation and acts as precursor for H_2O_2 and OH which cause wide range of free radical production in cellular compartments. Different endogenous antioxidants prevent free radicals' production during cellular activities and maintain redox state within cell in order to minimize oxidative stress. Such as neutralization of O_2 produce H_2O_2 which may be converted into nontoxic compound. SOD, GSH and ferritin are the major endogenous antioxidants [10].

Ferritin is an important endogenous antioxidants and it expropriate labialise iron. If endogenous antioxidants are not enough or unable to overcome oxidative stress exogenous antioxidants are needed to amplify antioxidant system of body [11]. Lack of various micronutrients are responsible for disorders which lead towards specific diseases[12],and it was observed that during the course of the disease, the demand for various nutrients also increases to maintain homeostasis and tissue repair. So supplementation of food additives increase the success rate of therapy as seen in TB[13].Oxidative stress increases MetHb percentage as Hb levels decreases during anemia .In this condition iron exist in its ferric state rather than ferrous state [14] .

Impairment in blood cells production (erythropoiesis) occurs when serum iron levels fall below 50.3 microgram /dL. A point reaches when iron stores deplete to the extent that they no longer support hemoglobin production and RBCs generation. This condition leads to anemia [15].

Vitamin C is the potential enhancer and antioxidant with physiological benefits and is of great importance due to its chelating property with iron and conversion of oxidation state of iron from insoluble state (Fe3+) ferric iron to soluble state (Fe2+) ferrous iron [16]. It forms complex with iron which is more absorbable in alkaline medium and hence easily taken up by small intestine[17].It contributes in redox homoeostasis and provides protection against reactive oxygen species and during oxidative stress helps in regeneration of vitamin A,E and glutathione [18].It is a major antioxidant present in brain to scavenge reactive oxygen species and is the main defense line of neuron cells[19].Few studies on iron and vitamin c were carried out which were conducted on human subjects

individually. But there is a lack of awareness about the synergistic effect of them in the correction of anemia, restoring maternal iron status and reduction of oxidative stress.

Material and Method

Study area:

This study was conducted in THQ Muridke, Pakistan from June 2023 to August 2023.

Sample size

The study sample was 40 participants (n=40).

Study groups

Patients were randomly divided into two groups G1 (Experimental group taking iron and vitamin c supplementation) and G2 (control group taking iron supplementation only).Nutritional counselling regarding intake of balanced diet and diet drug interaction was done to prevent the interaction of introduced supplements with its inhibitor and to ensure adequate nutritional intake . Each group has 20 participants.

Inclusion Criteria

- Lactating women of early lactation
- Subjects clinically diagnosed with iron deficiency anemia
- HCT(>35%)
- MCV (> 80microm^3)
- Serum Ferritin levels (>12 ng/ml)
- Serum Iron Levels(>50.3mcg/dl)
- Serum Vitamin C(>0.2mg/dl)
- Lactating women of age more than 18 years

Exclusion Criteria

- Pregnant women
- Uncorrectable bleeding
- Any inflammatory disease
- GIT cancer
- History of any psychological disorder.

Data collection Tools

Complete blood count CBC

After blood sampling, place the drawn sample in a vacuum tube with EDTA-K2 for anticoagulation and take three aliquots of blood (20 microliter).and put immediately in standard dilution solution. Measurements were carried out by automatic hematology analyzer within 1 hour of sampling [20].

Serum ferritin:

By using Electrochemiluminescence immunoassay, Serum ferritin is measured on Cobas e601, a sandwich principle which is completed in 18 minutes. Results were determined using a Calibrated curve [21].

Serum iron:

Determination of serum iron was performed at room temperature with 2 ml serum iron. Colored complex of iron was produced by using an ethanolic solution with specific ratio of 4;7 diphenyl - 1:10-phenanthroline suitable photometer of matched tubes with 1.1 cm inner diameter or 10-20mm fused glass cuvette was used for the measurement of optical density [22].

Serum vitamin C:
After blood sampling samples were immediately packed in a dark container with cooled temperature to prevent degradation of vitamin C. Determination of serum vitamin C was done by using HPLC kit and then analysis by means of shimadzu LC-20AT pump [23].

MetHb(%):
After blood sampling MetHb percentage was determined by spectrophotometric method four different wavelengths using double beam spectrophotometer. Distilled water containing cuvette was used as a blank. Hb absorbance was measured by subtracting blank absorbance from Hb absorbance at same wavelength [24].

TAC:
Automated method of total antioxidant capacity measurement was used for antioxidant assay .Hydroxyl radicals were produced when reagent 1 containing ferrous solution was mixed with hydrogen peroxide .And antioxidative effect of sample was measured against the free radical reaction initiated by hydroxyl radical [25].

Food Frequency Questionnaire (FFQ):
Food frequency questionnaire was used for the assessment of type and time of food consumption. Questionnaire was consist of a finite number of food and beverages. Frequency of each food enlisted was asked by the respondent and their responses were entered in a questionnaire.

Pictorial blood loss Assessment:
A visual scoring system was used to assess postpartum blood loss it consists of separate icons for both tampon and pad users which depict the amount of blood loss occurring and the responses of participants were recorded. After adding up the responses of the whole week, the amount of blood loss was calculated [26].

Ethical approval
This study was approved by the Research and Ethics committee of Riphah International University, Lahore.

STATISTICAL ANALYSIS
IBM SPSS Statistics 22.0 was used for the analysis of data. ShapiroWilk test was used to check normality of data and it was found that data was normally distributed. Paired T test was done for the analysis of variables within a group and independent T test for the analysis of variables between group. Demographic data was expressed and interpreted by percentage. $p<0.05$ was considered as a level of significance.

RESULTS
This study was designed for the evaluation of the synergistic effect of iron and vitamin c on CBC parameters, Serum iron, serum ferritin, serum vitamin C, MetHb% and TAC levels.

Baseline characteristics of participants
Age distribution of patients shows that in the control group 45% women were in between 18-24, 25%were in between 25-30 and 30%were in between 31-50. In experimental group 45% were in between 18-24, 45%in between 25-30 and 10% in between 31-50. Mode of delivery of patients in the control group was 95%Cesarean and 5% vaginal delivery. And in the experimental group 90% Cesarean and 10%vaginal delivery. Severity of anemia in the control group was 8% mild, 27% moderate and 65% severe. In experimental group 10% mild and 20%moderate and 70% severe. Inter delivery blood loss of all participants was less than 500ml so no one was suffering from postpartum

hemorrhage. Parity distribution in control group was 40% were primipara and 60% were para 2-4 and in experimental group 30% were primipara and 70% with para 2-4. In experimental group 30% participants intake fruit daily and 70% intake 2-3 times a month. Where in control group 45% participants' intake fruits daily while 55% intake 2-3 times a month. In experimental group 45% participants' intake dairy product regularly while 55% intake them irregularly in control group 40% participants intake dairy products regularly while 60% intake irregularly. Almost all participants intake chapatti rather than bread. In experimental group 35% participants intake meat and meat products once a week while remaining intake them once or twice a month. In control group 42% participants intake meat once a week while remaining intake it once or twice a month. All the participants of both groups experienced normal blood loss overall PBAC score of all the participants was (<90). Average CBC parameter of participants were Hb (7.3 to 7.5 g/dl), HCT (26.6 to 27%), MCV(64 fl),MCH(22 to 22.7pg),RBC count(3.6 to $3.9\times10^{6/}$µl)respectively, serum iron was(23.5 to 25.9 µg/dl),serum vitamin c (0.20 to 0.21 mg/dl) ,MetHb%(163 to 168%),TAC (1.46 to 1.49m mol Trolox Eq./L)and serum ferritin was(28.4 to 28.9 ng/ml).

Effect of Ascorbic acid and Iron supplementation on CBC parameters

Overall CBC parameters Hb, HCT, MCV, MCH of participants were increased significantly ($p<0.05$) in both groups but the results were more pronounced in experimental group treated with both vitamin c and iron supplementation .The highest increase was observed in experiment group from 7.8 to 13.7g/dl, 30.8 to 47%, 59 to 93fl and 24 to 32 pg respectively. However RBC count in participants of both groups did not increase significantly ($p>0.05$).

Effect of Ascorbic acid and iron supplementation on serum iron levels

Serum iron level of participants was noticeably increased ($p<0.05$) in both group but the improvement was more pronounced in those participants taking both vitamin c and iron supplementation. Whereas the highest increase was observed in experiment group from 47 to 102(µg/dl).

Effect of Ascorbic Acid and iron supplementation on Serum vitamin c levels

Serum vitamin C level of participants was noticeably increased ($p<0.05$) in both group but the improvement was more pronounced in those participants taking both vitamin c and iron supplementation. Whereas the highest increase was observed from 0.30mg/dl to 2mg/dl in the participant of experiment group.

Effect of Ascorbic Acid and iron supplementation on MetHb%:

MetHb % of the participants significantly decreased ($p<0.05$) in both groups but the decline in experimental group was more noticeable as compared to the participants of the control group. Whereas the maximum decrease was observed from 1.60% to1.2 % in experimental group.

Effect of Ascorbic Acid and iron supplementation on TAC:

TAC level of the participants of the experimental group significantly increased in ($p<0.05$) as compared to the participants of the control group. Whereas the highest increase was observed from 1.43 to 1.70 m mol Trolox Eq. /L I in experimental group.

Effect of Ascorbic Acid and iron supplementation on serum ferritin levels:

Serum ferritin levels of participants was noticeably increased ($p<0.05$) in both group but the improvement was more pronounced in those participants taking both vitamin c and iron supplementation. whereas the highest increase was observed in experimental group from 66 to 105 ng/ml.

Table 1: Effect of vitamin C and iron on CBC parameters, serum iron, serum ferritin, MetHb%, TAC and serum vitamin C of participants with postpartum anemia.

Parameters:	Group	Before treatment Mean±SD	After treatment Mean±SD
Hb(g/dl)	Experimental	7.3 ±0.69	13 ±0.82
	Control	7.5±0.85	10.9±0.9
HCT(%)	Experimental	26.6±2.8	38.2±2.9
	Control	27 ±4	34.6±2.3
MCV(fl)	Experimental	64 ±5.7	83.9±4.3
	Control	64 ±7.5	76.3±4.7
MCH(pg)	Experimental	22.7 ±3.2	28±1.3
	Control	22 ±3.8	24.9±2.3
RBC count($\times 10^6$/µl)	Experimental	3.9 ±0.3	4.6±0.23
	Control	3.6 ±0.49	4.5±0.27
Serum iron levels(µg/dl)	Experimental	25.6 ±9.8	100.4±9.08
	Control	23.5 ±8.4	74.5±13.22
Serum vitamin C(mg/dl)	Experimental	0.21 ±0.66	1.65±0.4
	Control	0.20 ±0.68	0.241±0.12
MetHb (%)	Experimental	1.68±0.07	0.97±0.04
	Control	1.63±0.65	1.3±0.35
TAC(m mol Trolox Eq./L)	Experimental	1.49±0.16	1.75±0.9
	Control	1.46±0.14	1.58±0.12
Serum Ferritin level(ng/ml)	Experimental	28.9±7	102.7±13.7
	Control	28.4 ±10.8	75.8±10.3

Fig 1:Pre &Post Hb of Participants(average)

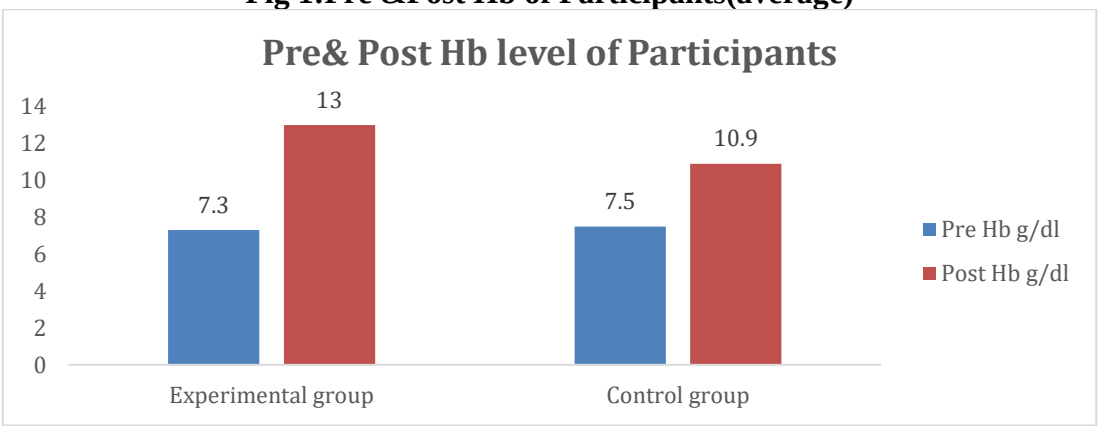


Fig 2:Pre & Post Serum Iron Level of Participant

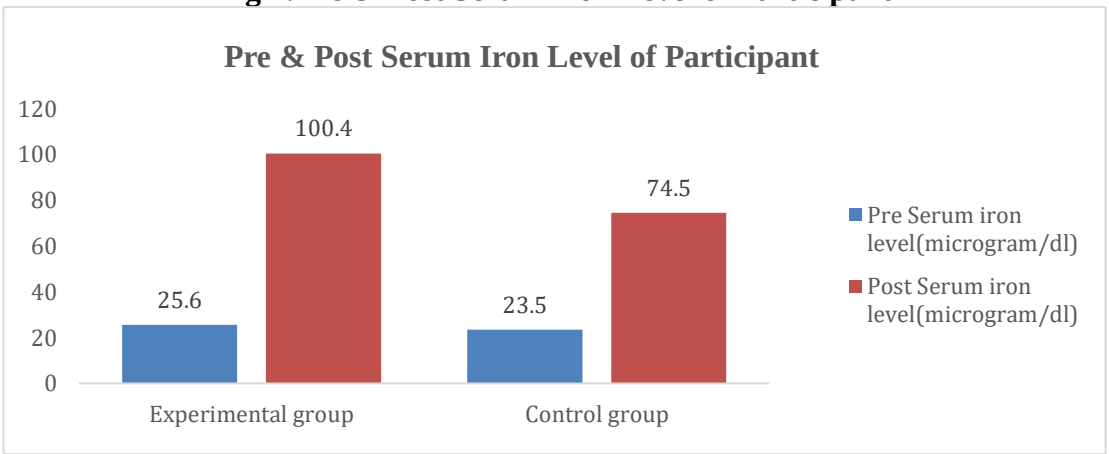


Fig 3:Pre&Post Serum Vitamin C Level of Participants

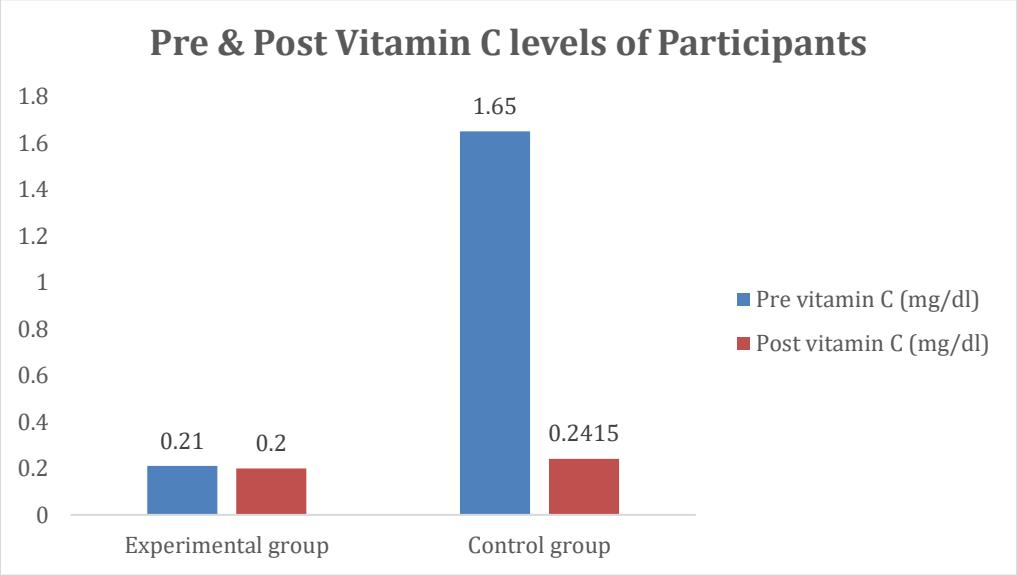


Fig 4:Pre&Post MetHb% of Participants

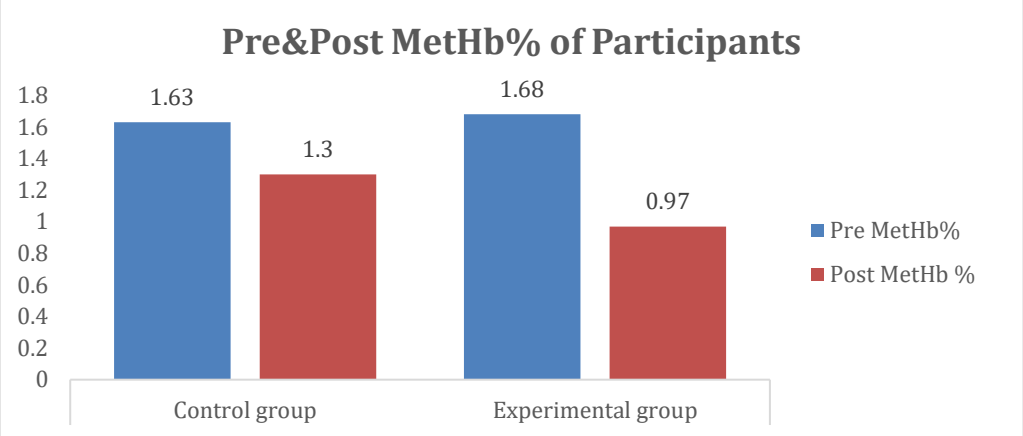


Fig 5:Pre&Post TAC level of Participants

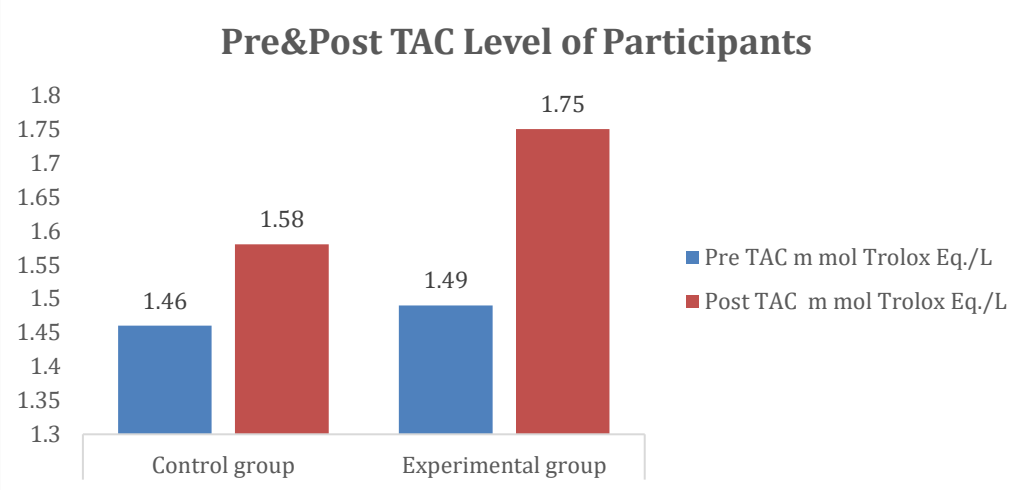
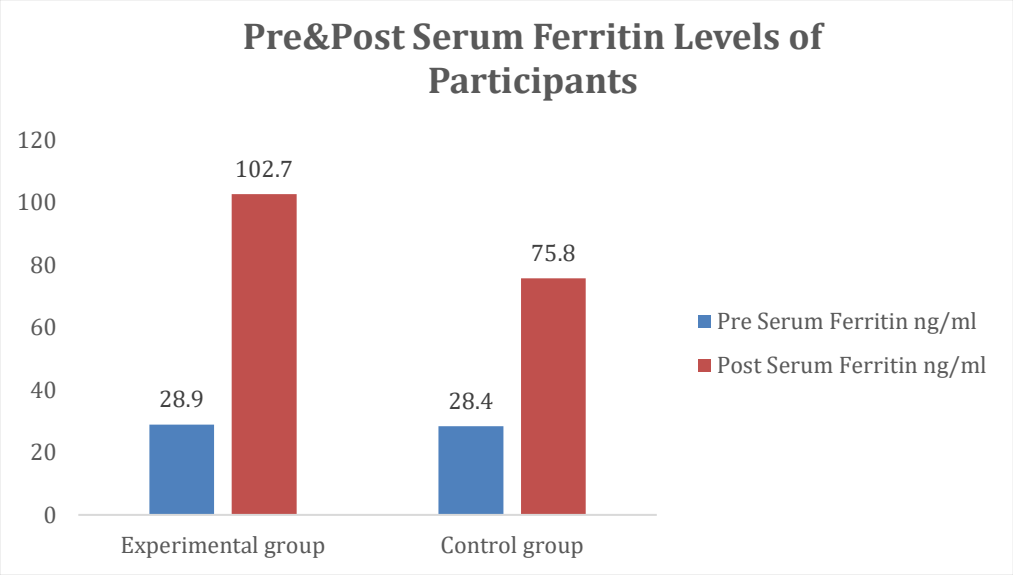


Fig 6:Pre&Post Serum Ferritin Level of participants



DISCUSSION

Current study was originated to assess the supplemental effect of Ascorbic acid, which is vitamin C along with iron in postpartum anemia, on maternal iron status and oxidative stress. And this study illustrated the positive effect of vitamin C and iron without exerting any major side effect. For this purpose, consumption of 500g of vitamin C and 100mg of iron poly maltose in supplemental form was administered to two groups for the period of 8 weeks in a single dose. In our case vitamin C and iron both increased Hb, MCV, MCH, HCT, serum vitamin Serum iron, TAC and serum ferritin levels significantly and decreased MetHb% significantly. Similar study was conducted to evaluate the effectiveness and safety levels of supplements of iron and vitamin C and it was concluded that MCV, MCHC, MCH levels increases in anemic patients taking supplementation of vitamin C with iron as compare to those having only iron intake [27].

And the result of current study was consistent with this research. RBC count was not significantly increased in both groups of the current study. A similar study was carried out in which it was concluded that iron deficiency persists for several weeks until normochromic normocytic cells are replaced by normal RBC population so effect of supplementation is not so rapid [28]. And the result of current study was consistent with this research .A research was conducted to evaluate the Redox Interactions of Vitamin C and Iron and concluded that conversion of oxidation state of iron from insoluble state (Fe^{3+})ferric iron to soluble state (Fe^{2+}) ferrous iron and neutralize acidic medium confirms the suitably of vitamin C to increase iron absorption [29].Result of current study was consistent with this research .Iron Polymaltose complex was use in the current study due to its effectiveness and less cost .A similar research was conducted to evaluate the comparison of effect, tolerability and pricing of two types of iron supplement one was iron polymaltose complex and the other on was iron ferrous sulfate in the treatment of IDA and it was stated that oral poly maltose complex is more effective more tolerable as well as more cost effective than ferrous sulfate [30].Result of current study was consistent with this study .Increased incidence of adverse effects were seen during supplementation of ferrous sulfate due to release of free radicals which facilitates damage and death of cells in iron polymaltose supplementation no free radical release this leads higher compliance and ensure regular treatment[31]. In current study it was evaluated that oxidative stress was higher in patients with anemia and assessment was done by MetHb % after supplementation MetHb percentage decreases significantly. Similar study was conducted on rates by feeding them iron deficit diet and after 9 weeks it was seen that Hb, Hct decreases and MetHb increases [14]. Result of both current and this study were consistent stating that oxidative stress plays a crucial role in denouement of anemia. Spectrophotometric method of MetHb assessment was used

in current as it has advantage over other methods due to small sample volume, less expensive, and accurate assessment. A similar study was conducted to assess Hb derivatives and this method of assessment was used for reliable results [24].

In experimental group participants were taking iron along with vitamin c which significantly increases blood parameters and decreases severity of anemia as compare to the group taking only iron .Similar observation was made in a study that antioxidant scavenge free radical form during cellular metabolism and if level of antioxidant decreases within body oxidation dominates and exogenous antioxidant needed to overcome this state. Vitamin c is a potential antioxidant and it also attenuate iron deficiency anemia [32] .Findings of both studies were consistent. It is not possible to evaluate individual antioxidant level in body so we measured total antioxidant capacity to predict the exact level of antioxidants and various methods are introduced to check TAC such as fluorescence and chemiluminescence but they are mostly not present in every laboratory.So an efficient and cheap method automated method was used in our study and it was seen that antioxidant levels of participants during pre-assessment were low. Similar study was conducted using similar method of TAC assessment and antioxidant levels were found low in anemic patients [25].In current study after the combine supplementation of vitamin C and iron serum iron and Hb levels of participant's increases. Similar research was conducted on Ascorbic acid and its impact of its absorption of iron in young women concluded that consuming diet with poor iron bioavailability ascorbic acid improve Hb and serum iron levels when supplementation of vitamin c along with iron continue for 5.5weeks [33].

These results are consistent with current study. In the current study participants were advised to take supplementation of both vitamin C (500mg) and iron (100mg) in a single dose and both at the same time. Similar research was conducted and concluded that facilitating impact of Vitamin C is more pronounced in a single meal as compared to complete diet. Multiple regression analysis showed relationship between ascorbic acid intake and iron absorption [34].Result of current study was consistent with this research. In Current study participants were advised to take vitamin c and iron supplements regularly for the period of 8 weeks and it was seen that hemoglobin and serum ferritin levels were significantly increased in them. Similar research was conducted to determine the effect of intermittent iron supplementation for reducing anemia and concluded that Hb level in intermittent supplementation was almost equal to daily dose but the level of ferritin was lower in intermittent dose than daily[35].In current study therapeutic effect was seen in the group taking both iron and vitamin c supplementation .Similar study was conducted Postpartum anemia as an health issue and concluded that Vitamin C intake greatly increases iron absorption and this association give positive results as it was seen that higher risk of anemia was seen in patients with low vitamin C intake [36].And the result of current research was consistent with research. Previous studies and literature support the outcomes of the current study that supplementation of vitamin c along with iron significantly improves CBC parameters, serum iron levels, serum ferritin levels, TAC, MetHb% and serum vitamin c levels of participants.

CONCLUSION

Based on the literature and current study it is concluded that Supplementation of iron and vitamin c improves Hb, MCV, HCT, MCH, TAC, Serum vitamin C, serum ferritin, serum iron levels and decreases MetHb% significantly as compared to control group, but improvement in RBCs count was not found significant. As in this combination absorptive rate of non heme iron was increased and oxidative stress was significantly decreased by Vitamin C. Hence both Synergistic and antioxidant effects were observed.

Conflict of Interest Statement:

Authors proclaimed no conflict of interest

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