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A STUDY OF EFFECTS OF VITAMIN C & VITAMIN E SUPPLEMENTATION ON NUTRITIONAL ANAEMIA IN TERTIARY CARE HOSPITAL

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Abstract

Objective: Nutritional anaemia, one of the most common global health concerns, is associated with an increase in free radicals. Antioxidants, such as vitamin C & vitamin E are known for their beneficial effects on oxidative stress. The study was conducted with the aim of studying the combined effects of vitamins C & E on the treatment of iron deficiency anaemia (IDA). **Materials & Methods:** A total of 140 patients with known IDA were studied in the Department of Physiology Santosh Medical College in collaboration with the Department of Medicine Sharda Hospital. These patients were divided into 2 groups each of 70 per group. The control group was treated with ferrous sulphate and the test group was treated with ferrous sulphate, vitamin C and vitamin E. Complete blood counts of all the patients were measured before and after treatment and the data was statistically analysed. **Results:** Patients in both groups demonstrated statistically significant improvement in their Hb, MCV, and MCHC levels, suggesting improvement in IDA. This improvement was higher in patients taking Vitamin C & E compared to the patients taking ferrous sulphate alone. Haematological parameters, such as TIBC, Serum Ferritin and RDW also established a better improvement in iron storage in patients taking antioxidants. **Conclusion:** The study demonstrated that administrating antioxidants, in conjunction with ferrous sulphate, in the treatment of IDA could provide better results.

Keywords: Iron Deficiency Anaemia, Antioxidants, Vitamin C, Vitamin E.

INTRODUCTION

Anaemia, one of the major health concerns worldwide, is a condition in which the number of red blood cells, and consequently blood's oxygen-carrying capacity, is insufficient to meet the physiological demands of the body. Anaemia is defined as Hemoglobin (Hb) levels of less 13 g/dL in adult men, and Hb levels of 12 g/dL in adult non-pregnant women [(1)]. Anaemia can be caused by either decreased production or increased loss of Red Blood cells and can be categorised according to its cause. Nutritional anaemia is one of the common causes of anaemia and develops due to deficiency of iron, folate or vitamin B12. Iron Deficiency anaemia (IDA) is recognised by WHO as the most common nutritional deficiency in the world, affecting 30% of the population[(2)]. IDA is associated with prematurity and low birth weight during pregnancy, defects in cognitive and psychomotor development during childhood, and impaired work capacity in adulthood[(3)]. IDA is commonly treated with iron supplementations, such as ferrous sulphate. A reactive oxygen species (ROS) is an oxygen containing molecule with an unpaired electron and is capable of damaging biologically relevant molecules, such as protein and DNA. The detrimental effects of free radicals can be neutralized by antioxidant molecules synthesized in our body, like glutathione, uric acid, or ubiquinone, and those obtained from the diet, such as vitamins C, E, and A, and flavonoids[(4)]. Vitamin C (ascorbic acid) is a water-soluble

vitamin and acts as a co-factor in a variety of biological functions such as collagen synthesis. Vitamin E is a fat-soluble vitamin, particularly important for the protection of cell membranes as well as for keeping our skin, nerves, and red blood cells healthy.

Oxidative stress develops when there is an increase in ROS and/or impairment of the antioxidant mechanisms. Studies have reported that oxidative stress is one of the potential biochemical mechanisms involved in the pathogenesis of iron deficiency anaemia[3]. This research conducted to study the effects of antioxidants (Vitamin C & E) on complete blood count in patients with nutritional anaemia (IDA) when they are treated with ferrous sulphate.

MATERIALS & METHODS

An interventional and cross-sectional study was conducted in the Department of Physiology, Santosh Medical College in collaboration with Department of Physiology & Department of Medicine, School of Medical Sciences and Research, Sharda Hospital, Greater Noida for a duration of two & half years from November 2017 to April 2020. The study comprised of 140 patients with known IDA in the age group of 18-60 years. Following haematological criteria were used to identify patients with IDA.

- Haemoglobin - adult men and women with Hb <13 and < 12 mg/dL respectively,
- Mean corpuscular volume (MCV) <80 fL,
- Mean corpuscular haemoglobin concentration (MCHC) <32 g/dL
- Serum Ferritin <30 ng/L
- Total iron binding capacity (TIBC) >450 mcg/dL
- Red cell distribution width (RDW) >14%

Patients were subdivided into 2 groups i.e. control group and test group. Each group consisted 70 patients with no family history of anaemia. Details of the study had been given to patients & a written Informed Consent from willing patient was taken & procedure was explained. Patients were randomly divided every odd number was allotted to Control group & even numbers were allotted to Test group. The patients in the test group were given Tablet Limcee (Vitamin C) of 500 mg and Tablet Evion (Vitamin E) of 400 mg. Patients in both the groups were prescribed ferrous sulphate 200 mg. All the supplements were given empty stomach daily once a day for 12 weeks. Patients were told to stop medication in case of adverse reactions like any allergic reaction etc & to investigate immediately.

Clinical Parameters & cut-off values

Venous blood sample (as per requirement) was collected with complete aseptic precautions over the duration of the study. Complete blood count (Haemoglobin, red blood cell count, MCV, MCH, MCHC) were done by haematology cell counter (SYSMEX, XP100 cell counter).

Data Collection

The data on the clinical parameters were gathered in 2 phases i.e. pre-supplementation and post-supplementation of Vit C & Vit E. In pre supplementation phase: After preliminary discussions with the patients of both the groups regarding the study, their blood count was recorded. Then supplementation was recommended to

each patient in the test group in the presence of physician along with the prescribed treatment.

- Dose and method of consuming the supplementation of antioxidants (vitamin C& E) were explained briefly to the patients.
- The patients were asked to revisit the clinic/OPD after 12 weeks.
- Telephone contacts during the supplementation phase was made to ensure the regular intake of supplementation in prescribed format.

In post supplementation phase: Patients from both the groups were asked to revisit the clinic after 12 weeks and repeat their complete blood count. The collected data was analysed by using IBM-SPSS version 22.0.

Statistical Analysis

Data analysis was done as follows:

1. Variation in the levels of haemoglobin, MCV, MCHC, and RBC count of anaemic subjects in the test and control groups was studied
2. All the values were analysed statistically based on student's (t) test and 'P' values.

OBSERVATION AND RESULTS

Out of 140 total subjects, about 65.7% were males and 34.3% were females. In the control group, the mean value of Haemoglobin (HB) was 9.23+1.64 g/dL at the beginning of the study and 9.43 +1.98 g/dL at the end of 12 weeks [Table 1]. While in the test group, the mean Hb in pre supplementation phase was 9.23 +1.68 g/dL and in the post-supplementation reading, it was 10.46+1.69 g/dL [Table 2]. The difference in Hb levels between pre and post supplementation phase was statistically significant in both the groups. Improvement was observed in the values of MCV, MCHC, RBC, and serum ferritin count between the pre and post supplementation phase in both the groups, and it was significantly higher in the test group. A significant reduction was detected in total iron binding capacity and red cell distribution width in both the groups, indicating improvement in serum iron levels.

Table 1: Comparison of complete blood counts parameters in anaemic patients of control group

	Control group (1st reading) Mean ± SD	Control group (2nd reading after 3 months) Mean ± SD	P value
Haemoglobin (g/dL)	9.23±1.64	9.43 ±1.98	0.01
PCV (%)	24.66± 3.8	25.98±3.11	0.13
MCV(fl)	74.78± 9.73	75.96 ±9.19	0.03
MCH (pg)	23.71 ±3.26	23.75± 3.02	0.02
MCHC (gms %)	30.1 ±3.83	31.6 ±3.25	0.02
RBC Count (million/uL)	3.46± 0.45	3.79±0.41	0.04
Serum Ferritin (ng/L)	27.46± 1.23	28.98± 1.38	0.02
TIBC (mcg/dL)	478± 29.36	428± 28.12	0.04
RDW (%)	14.9± 0.64	14.23 ± 0.59	0.03

*p value<0.05 is statistically significant

Table 2: Comparison of complete blood counts parameters in anaemic patients of test group

	Test group (1st reading) Mean ± SD	Test group (2nd reading after 3 months) Mean ± SD	P value
Haemoglobin	9.23 ±1.68	10.46 ±1.69	0.02
PCV (%)	24.98±3.11	26.97 ±4.35	0.01
MCV(fl)	73.96 ±9.19	76.46 ±9.19	0.03
MCH (pg)	23.75± 3.02	26.35 ±4.02	0.01
MCHC (gms %)	30.8 ± 2.95	31.7± 4.25	0.01
RBC Count (million/uL)	3.52 ±0.41	4.01± 0.50	0.01
Serum Ferritin (ng/L)	26.90 ± 1.15	29.10 ± 1.29	0.03
TIBC (mcg/dL)	470± 30.16	415± 29.22	0.02
RDW (%)	14.87± 0.61	14.18 ± 0.58	0.03

*p value<0.05 is statically significant Abbreviations:

PCV: Packed Cell Volume

MCV: Mean corpuscular volume

MCHC: Mean corpuscular haemoglobin concentration RBC count: Red blood cell count

TIBC: Total iron binding capacity RDW: Red cell distribution width

IDA is a type of hypochromic, microcytic anaemia with low iron storage in the body. Improvement in the values of MCV and MCHC suggest increase in the red blood cell size and improvement of Haemoglobin concentration in erythrocytes respectively. Iron deficiency also leads to increase in the total iron binding capacity (TIBC) and red cell distribution width (variation in red cell size). Their post-implementation levels in the test group compared to the control group, suggest that patient taking antioxidants also had enhanced iron storages.

DISCUSSION

One of the most common causes of the anaemia in the world, especially in the developing nation is nutritional anaemia. It effects million of the people, especially young children and pregnant women. The global prevalence of the of anaemia is 29.6% in women and 39.8% in children aged 6-59 months[5]. It is estimated that IDA is responsible for half of the global prevalence of anaemia[6].

Iron is an essential molecule in haem metabolism, and deficiency of iron results in microcytic and hypochromic anaemia. Causes of iron deficiency may vary. Insufficient iron intake, decreased absorption of iron from GIT or increased blood loss, are usually a few common reasons. Most patients with IDA are asymptomatic, and identified through routine check-ups. Symptoms of IDA may include fatigue, shortness of breath or decreased work ability. Severe IDA can lead to developmental delays in children, increased risk of infections, or complications in pregnancy. Iron supplementation, such as Ferrous sulphate, are commonly used for the treatment of IDA.

Free radicals are molecules with at least one unpaired electron it the outer membrane and with high reactivity. Free radicals can be derived from oxygen (Reactive oxygen species – ROS) or nitrogen (Reactive nitrogen species – RNS). ROS play crucial role in various biological activities, including cellular proliferation and apoptosis. In contrast, excessive ROS may lead to damage to important biomolecules, such as DNA, proteins

and membranes[7]. An antioxidant is a reducing agent, which has the capability to inhibit oxidation of other molecules. Antioxidants have extra electrons and can donate them to the free radicals, resulting in their neutralization. Oxidative stress is an imbalance between the production of free radicals and antioxidants defense of the body.

Molecules synthesized by the body, such as glutathione, uric acid, or ubiquinone and obtained from the nature, such as vitamins C, E, and A, and flavonoids act as antioxidants(4). Vitamin C (Ascorbic acid) and vitamin E are common vitamins available in the natural food sources such as fruits, berries, oranges and nuts, seeds, green leafy vegetables respectively. Vitamin C is effective in prevention of non-enzymatic glycosylation of proteins is demonstrated to be an important factor in lipid regulation. Vitamin E is particularly important for the protection of our cell membranes as well as keeping our skin, heart and circulation, nerves, muscles and red blood cells healthy.

In the pathogenesis of IDA, increased oxidative stress might be one of the contributing factors and the severity of IDA might be associated with the level of oxidative stress[3]. Several studies have reported that oxidative/ antioxidant balance shifted towards the previous in the IDA patients[8, 9]. A few studies have demonstrated that patients with IDA are subject to chronic oxidative stress[10]. As oxidative stress is associated with the pathogenesis of IDA, use of antioxidants in the IDA treatment can be beneficial to study. This research was aimed to evaluate the relationship between treatment of IDA and benefits of using antioxidants during the treatment period. The study was conducted in the Department of Physiology, Santosh Medical College in collaboration with Department of Physiology & Department of Medicine, School of Medical Sciences and Research, Sharda Hospital, Greater Noida for a duration of two & half years. A study population of 140 patients were divided into two groups – control and test group - with 70 patients each. Ferrous sulphate 200 mg, one of the most widely used drug for IDA was used for the treatment of IDA in patients of both the groups. To evaluate the effects of antioxidants, the test group was also treated with Tablet Limcee (Vitamin C) of 500 mg and Tablet Evion (Vitamin E) of 400 mg. The treatment was given for the duration of 12 weeks. Complete blood count, including Hb, MCV, MCHC and red blood cell count, was calculated before and after the implementation of the treatment in all the subjects.

On an average, patients in both the groups experienced improvement in their complete blood count levels. In the control group, the mean value of Haemoglobin (HB) was 9.23+1.64 g/dL at the beginning of the study and 9.43 +1.98 g/dL at the end of 12 weeks. RBC count also showed an improvement from 3.46+ 0.45 to 3.79+0.41 (million/uL). In the test group, the mean Hb in pre supplementation phase was 9.23 +1.68 g/dL and in the post-supplementation reading, it was 10.46+1.69 g/dL. RBC count was increased from 3.52 + 0.41 to 4.01+ 0.50 (million/uL). Also, Improvement was observed in the values of MCV, MCHC, PCV, and serum ferritin count between the pre and post supplementation phase in both the groups, and it was significantly higher in the test group. Mean value of total iron binding capacity and red cell distribution width was reduced in both the groups, but more significant reduction was observed in the test group. All the results were analysed by using IBM-SPSS version 22.0 and p value <0.05 was considered statically significant.

The results of the study showed that the patients who are taking ferrous sulphate plus antioxidants such as Vitamin C & E, experienced an overall better improvement in their Hb levels compared to the patients who were not taking antioxidants. The difference of the improvement in both the groups was also statistically significant. The values of TIBC, Ferritin and RDW suggest that improvement in iron levels was better in the subjects taking antioxidants, in comparison with patients taking just ferrous sulphate. The results of this research were in accordance with the hypothesis of the research, that using antioxidants in the treatment of the IDA, can ensue better improvement of the Hb levels in IDA patients.

CONCLUSION

In light of the findings, we concluded that in the patients of IDA, antioxidant vitamins in conjunction with ferrous sulphate, can produce better haematological results than ferrous sulphate alone. Supplementation of combined doses of Vitamin C & E, acted as antioxidants and help reduce the oxidative stress in the patients. Although, further research might be required to measure serum total antioxidant capacity, serum total peroxide level and oxidative stress index before and after the administration of the antioxidants to have a better understanding of their effects. Research studies with longer duration & higher doses of vitamins can give further clarification.

Ethical clearance: Obtained from college and hospital ethical committee.

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