Comparative studies on Iron deficiency anaemia in women of reproductive age groups in Taldangra block of Bankura, West Bengal

Thesis Submitted to Vidyasagar University for the Partial Fulfillment of the Degree of Master of Medical Laboratory Technology

Submitted by

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Date: 13.07.2023

CERTIFICATE

This is to certify that the project report entitled "**Comparative studies on Iron deficiency anaemia in women of reproductive age group in Taldangra block of Bankura, West Bengal**" submitted by Abir Banerjee [Roll-PG/VUWGP29/MLT-IVS No.-001], Akash Sinha Mahapatra [Roll-PG/VUWGP29/MLT-IVS No.-002], Animesh Chakraborty [Roll-PG/VUWGP29/MLT-IVS No.-004], Anindya Ghosh [Roll-PG/VUWGP29/MLT-IVS No.-005], Arpan Hait [Roll-PG/VUWGP29/MLT-IVS No.-006], Arup Mahanti [Roll-PG/VUWGP29/MLT-IVS No.-007], Avishek Kar [Roll-PG/VUWGP29/MLT-IVS No.-010], Bhabatosh Mahata [Roll-PG/VUWGP29/MLT-IVS No.-011], Debnath Bir [Roll-PG/VUWGP29/MLT-IVS No.-012], Dipak Sarkar [Roll-PG/VUWGP29/MLT-IVS No.-015] to the Midnapore City College, Midnapore, West Bengal, India during the year of 2022 in partial fulfillment for the award of the degree of M.Sc. in Medical Laboratory Technology is a bona fide record of project work carried out by him/her under my/our supervision. The contents of this report, in full or in parts, have not been submitted to any other Institution or University for the award of any degree.

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Declaration

We do hereby declare that the present Master thesis entitled '**Comparative studies on Iron deficiency anaemia in women of reproductive age group in Taldangra block of Bankura, West Bengal**' embodies the original research work carried out by me in the Department of Biological Sciences, Midnapore City College, Paschim Medinipur, West Bengal, India under the supervision of **Mrs. Monalisha Karmakar**, Assistant Professor, Paramedical & Allied Health Science, **Midnapore City College**, Kuturiya, Bhadutala, Pin-721129. No part thereof has been submitted for any degree or diploma in any University.

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Dedicated to beloved Parents and Teachers

Acknowledgement

We would first like to acknowledge Dr. Pradip Ghosh, Hon'ble Founder Director, Midnapore City College, Paschim Medinipur for providing me the opportunity to study and complete my thesis work in this college. I am gratefully indebted to him for his very valuable comments on this thesis.

We would like to thank my thesis advisor Mrs. Monalisha Karmakar of the Paramedical & Allied Health Science at Midnapore City College. The door to Prof. Karmakar office was always open whenever I ran into a trouble spot or had a question about my research or writing. She consistently allowed this paper to be my own work but steered me in the right the direction whenever he/she thought I needed it.

We would also like to thank the other Faculties (Dr. Kuntal Ghosh, Dr. Shantanu Kar Mahapatra, Mr. Subhadeep Mandal, Mr. Sourya Sekhar Mitra, Mr. Surya Kanti Dey, Ms. Shilpa Sarkhel) and stuffs (Mr. Pintu Manik and Mr. Pathik Laha) for their support to carry out this research project. Without their passionate participation and input, the validation survey could not have been successfully conducted.

Finally, We must express my very profound gratitude to my parents for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this thesis. This accomplishment would not have been possible without them.

Thank you.

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INTRODUCTION

Introduction

The term ‘Anaemia’ refers to ‘a reduction below normal in the concentration of haemoglobin or red blood cells in blood’. Anaemia may be regarded in physiological term as ‘reduction in oxygen transporting capacity of blood (Godkar and Godkar, 2003). It is a condition in which the number of red blood cells (RBCs), and consequently their oxygen-carrying capacity, is insufficient to meet the body’s physiological needs. The function of the RBCs is to deliver oxygen from the lungs to the tissues and carbon dioxide from the tissues to the lungs. This is accomplished by using haemoglobin (Hb), a tetramer protein composed of haem and globin. Anaemia impairs the body’s ability for gas exchange by decreasing the number of RBCs transporting oxygen and carbon dioxide. Anaemia results from one or more of the following processes: defective red cell production, increased red cell destruction or blood loss. Iron is necessary for synthesis of haemoglobin.

Iron deficiency anaemia remains the most common cause of anaemia not only in India but also all over the world. According to the World Health Report, there are 1,788,600 people in this world suffering from iron deficiency anaemia and iron deficiency anaemia is the foremost prevalent disease-causing morbidity in the world (WHO, 1998). Other nutritional deficiencies (including folate, vitamin B12 and vitamin A), acute and chronic inflammation, parasitic infections, and inherited or acquired disorders that affect Hb synthesis, red blood cell production or red blood cell survival can all cause anaemia. Iron deficiency anaemia results in impaired cognitive and motor development in children and decreased work capacity in adults. The effects are most severe in infancy and early childhood. In pregnancy iron deficiency anaemia can lead to perinatal loss, prematurity and low birth weight (LBW) babies. Iron deficiency anaemia also adversely affects the body’s immune response.

Aetiology of Anaemia

The commonest causes of anaemia in developing countries, particularly among the most vulnerable groups (pregnant women and preschool age children), are nutritional disorders and infections. Hence the causes of anaemia could be segregated as nutritional and non-nutritional, underscoring the aetiological importance of dietary deficiency as the major causative factor.

Iron deficiency

Iron status can be considered as a continuum from iron deficiency with anaemia, to iron deficiency with no anaemia, to normal iron status with varying amounts of stored iron, and

finally to iron overload which can cause organ damage when severe. Iron deficiency is the result of long-term negative iron balance. Iron deficiency anaemia (IDA) should be regarded as a subset of iron deficiency, that is, it represents the extreme lower end of the distribution of iron deficiency.

Iron deficiency adversely affects-

- The cognitive performance, behaviour and physical growth of infants, preschool and school-age children.
- The immune status and morbidity from infections of all age groups.
- The use of energy sources by muscles and thus the physical capacity and work performance of adolescents and adults of all age groups.

Adolescence is an opportune time for interventions to address anaemia, as it is an important time of growth and development. Missing out on nutrition education and IFA supplementation at this time may push young boys and girls further into the cycle of iron deficiency and anaemia. In adolescent girls, apart from meeting growth needs, sufficient iron intake is also essential before and during pregnancy.

Iron requirements are highest for pregnant women –1.9 mg/1,000 Kcal of dietary energy in the second trimester and 2.7 mg/1,000 Kcal in the third trimester. These are followed by iron requirements in infants (1.0 mg), adolescent girls (0.8 mg), adolescent boys (0.6 mg), non-pregnant women (0.6 mg), preschool and school age children (0.4 mg), and adult men (0.3 mg).

- Iron deficiency is a consequence of:
- Decreased iron intake
- Increased iron loss from the body
- Increased iron requirement

Iron requirements increase during the period of active growth in childhood, especially from 6 months to 3 years. In infancy, iron deficiency is most often the result of lack of exclusive breastfeeding and use of unsupplemented milk diets which contain inadequate amounts of iron. Milk products are very poor sources of iron and prolonged breast or bottle feeding of the infant without complementary feeds after 6 months of age frequently lead to iron deficiency unless there is iron supplementation. Iron requirements are proportionately greater in premature and underweight babies. In older children, a predominantly milk and cereal based diet and food fads can also lead to IDA.

Blood loss during menstruation and increased iron requirements during pregnancy and lactation predispose women to poor iron stores. Traditionally, the Indian housewife eats last, after all male members and children have eaten and, in many families, the women eat only the leftovers. Hence, even though the food prepared for the family is the same, women are more prone to develop IDA than other members of the family.

Other micronutrient deficiencies like Vitamin B_{12} is necessary for the synthesis of RBCs and its deficiency has been associated with megaloblastic anaemia. Diets with little or no animal protein, as is often the case in our country, coupled with malabsorption related to parasitic infections of the small intestine, might result in Vitamin B12 deficiency and anaemia. Folic acid is also essential for the formation and maturation of RBCs and is necessary for cell growth and repair. Deficiency of folate reduces the rate of DNA synthesis with consequent impaired cell proliferation and intramedullary death of resulting abnormal cells; this shortens the lifespan of circulating RBCs and results in anaemia. Helminths such as hookworm and flukes cause chronic blood loss and consequently iron loss from the body, resulting in the development of anaemia. A hookworm burden of 40–160 worms (depending on the iron status of the host) is associated with IDA. Malaria, especially by the protozoa Plasmodium falciparum and vivax, causes anaemia by rupturing RBCs and suppressing production of RBCs. Decreased RBC production results from marrow hypoplasia seen in acute infection. Malaria in pregnancy increases the risk of maternal anaemia, stillbirth, spontaneous abortion, LBW and neonatal deaths. Certain chronic diseases, such as cancer, HIV/AIDS, rheumatoid arthritis, Crohn's disease and other chronic inflammatory diseases, can interfere with the production of RBCs, resulting in chronic anaemia.

REVIEW OF LITERATURE

Review of Literature

Global Overview

According to the World Health Report, there are 1,788,600 people in this world suffering from iron deficiency anaemia. And iron deficiency anaemia is the foremost prevalent disease-causing morbidity in the world (WHO, 1998). Anaemia is estimated to contribute to more than 115,000 maternal deaths and 591,000 perinatal deaths globally per year (Ezzati et al., 2004). Analysis of data on global prevalence shows that anaemia is disproportionately concentrated in low socioeconomic groups, and that maternal anaemia is strongly associated with child anaemia.

The WHO Global Database on Anaemia for 1993–2005, covering almost half the world's population, estimated the prevalence of anaemia worldwide at 25 per cent (WHO, 2008). In absolute numbers anaemia affects 1.62 billion people globally with about 293 million children of preschool age, 56 million pregnant women, and 468 million non-pregnant women estimated to be anaemic (WHO, 2008). Although the prevalence of anaemia is estimated at 9 per cent in countries with high development, in countries with low development the prevalence is 43 per cent (McLean et al., 2009).

Children and women of reproductive age are most at risk, with global anaemia prevalence estimates of 47 per cent in children younger than 5 years, 42 per cent in pregnant women, and 30 per cent in non-pregnant women aged 15–49 years (McLean et al., 2009). Africa and Asia account for more than 85 per cent of the absolute anaemia burden in high-risk groups and India is the worst hit.

In a study of 85 men and 54 women in Finland, only traces of marrow iron were found in 4 to 7% of men, in 70% of women of 15 to 49 years of age, and in 23% of women of 50 years of age or older (Takkunen, 2009). In a survey of 1105 Canadians, iron stores, judged by serum ferritin values, were greatly reduced in about 25% of children, 30% of pregnant women, and 3% of men (Valberg et al., 2009). In a study, it was found that 9% of toddlers aged up to 2 years, 9% to 11% of adolescent girls, and women of childbearing age were found to be iron deficient. Of these, iron deficiency anaemia was found in 3% and 2% to 5%, respectively (Looker et al., 2009).

Indian Scenario

India is one of the countries with very high prevalence of anaemia in the world. Almost 58 per cent of pregnant women in India are anaemic and it is estimated that anaemia is the underlying cause for 20–40 per cent of maternal deaths in India. India contributes to about 80 per cent of the maternal deaths due to anaemia in South Asia (Ezzati et al., 2002).

Nutritional anaemia is a major public health problem in India and is primarily due to iron deficiency. The National Family Health Survey-3 (NFHS-3) data suggests that anaemia is widely prevalent among all age groups and is particularly high among the most vulnerable nearly 58 per cent among pregnant women, 50 per cent among non-pregnant non-lactating women, 56 per cent among adolescent girls (15–19 years), 30 per cent among adolescent boys and around 80 per cent among children under 3 years of age which is shown in the following (Table 1).

Table 1: Prevalence of anaemia among different age groups

Age groups	Prevalence of anaemia (%)
Children (6–35 months)	79
Children (6–59 months)	69.5
All women (15–49 years)	55.3
Ever married women (15–49 years)	56
Pregnant women (15–49 years)	58.7
Lactating women (15–49 years)	63.2
Adolescent Girls	
12–14 years	68.6*
15–17 years	69.7*
15–19 years	55.8

Source: NFHS-3

Prevalence of anaemia among adolescent girls

The prevalence of anaemia among girls (Hb <12 g%) is alarmingly high as per the reports of NFHS-3 and the National Nutrition Monitoring Bureau Survey (NNMBS). There are over 55 per cent of adolescent girls are anaemic. Percentage prevalence of anaemia among adolescent girls in the age group 15–19 years and in the older age group 20–29 years remains almost stagnant at 55.8 per cent and 56.1 per cent respectively.

Prevalence of anaemia among pregnant women, men and women of reproductive age (WRA)

Anaemia is a major health problem for adults as well, affecting 55 per cent of women, 58 per cent of pregnant women. The prevalence of anaemia among ever married women increased from 52 per cent in NFHS-2 to 56 per cent in NFHS-3.

Anemia in women of reproductive age (15–49 years of age) in low- and middle-income countries (LMICs) continues to be an intractable problem of 'hidden hunger', exemplifying gender health inequities and a shameful loss of human capital. The low hemoglobin concentration in blood that defines anemia occurs long after tissue iron stores have been depleted to levels associated with suboptimal function. A major cause of anemia, this iron deficiency leads to diminished oxygen-carrying capacity in red blood cells, which in turn diminishes energy efficiency, work capacity and productivity (Haas and Brownlie 2001). In addition, severe anemia in pregnancy is consistently linked to maternal mortality (Lammi-Keefe 2008). Anemia in women of reproductive age is concentrated in LMICs and requires urgent and immediate attention. In this issue of Nature Medicine, Kinyoki and colleagues set out to model the prevalence and burden of mild, moderate and severe anemia in LMICs over the past two decades (Kinyoki et al., 2018). The study examines data from several databases across 82 LMICs, comprising 218 geo-referenced household surveys conducted between 2000 and 2018 and including over three million women. On the basis of their analysis, the authors estimate half a billion women were living with anemia in LMICs in 2018. In the period from 2000 to 2018, they find limited decreases in anemia prevalence (35.6% to 31.6%) and an increase in years lived with disability, due to population growth. They reveal widespread sub-national disparities and predict that a vast majority of countries will fail to achieve the World Health Organization global nutrition target of a 50% reduction in anemia prevalence by 2030. Although limited by data quality and quantity gaps, this novel analysis provides an opportunity to shed light, anew, on an important public-health problem and public-policy failure.

AIM AND OBJECTIVES

Aim and Objectives

Anaemia has been reported to be a major micronutrient deficiency among Adolescent girls across the country (Menon et al., 2014). On the other hand, Iron deficiency is the most common nutritional disorder in the developing world and the most common cause of nutritional anaemia in women of reproductive age (Kotecha et al., 2009).

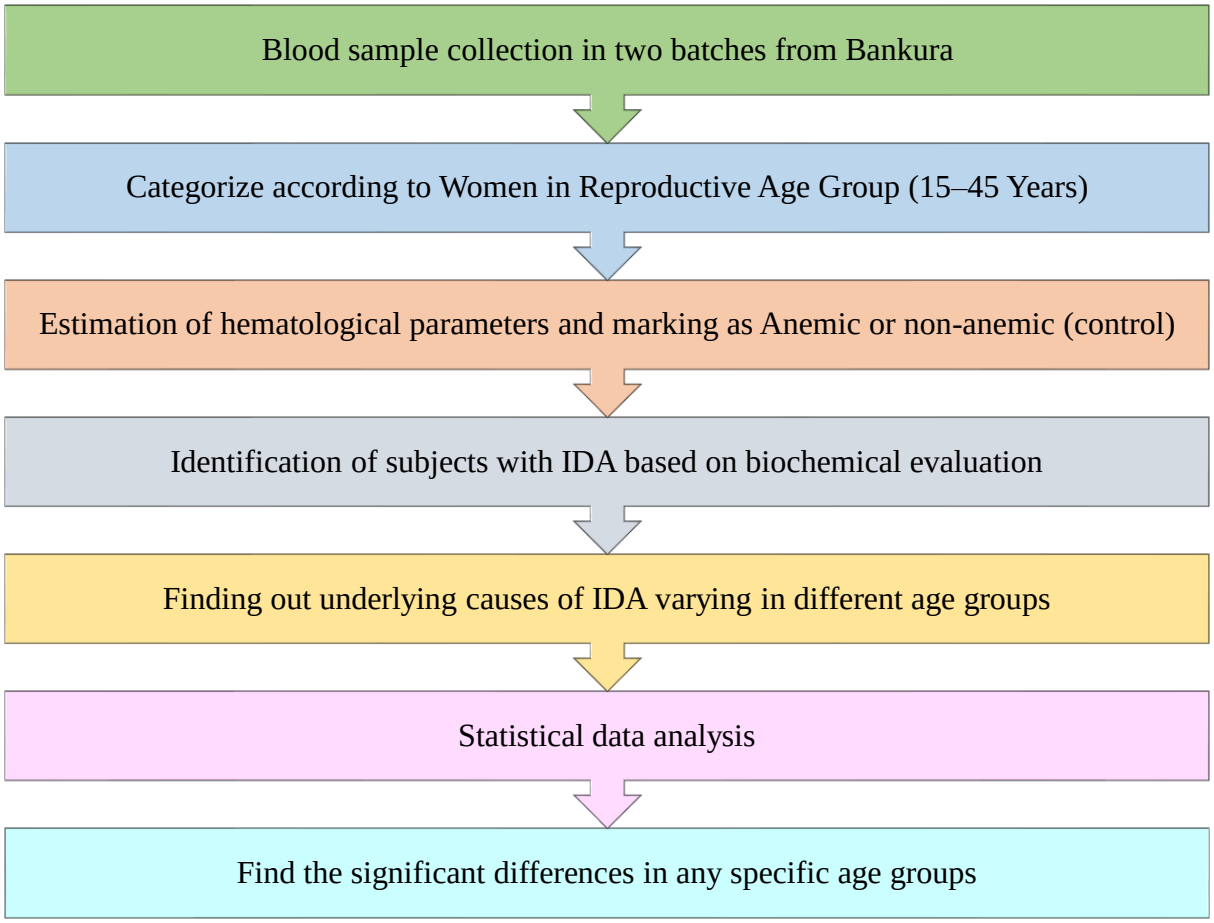
So, the main aim of this study is to develop awareness regarding Iron deficiency anemia in the women with reproductive age group (WRA)i.e. 15-45 years.

It has few objectives also i.e.-

- Differentiate the anemic subjects from the non-anemic women.
- Detection of iron deficiency anemic (IDA) subjects on the basis of biochemical factors.
- Comparative analysis of hematological parameters in each age group of IDA subjects.
- Evaluation of the age group of women, who are prone to suffer much in IDA.

EXPERIMENTAL DESIGN

Experimental Design



MATERIALS AND METHODS

Materials and methods

- **Study Design**: A cross-sectional study was conducted in Taldangra block of Bankura, West Bengal and the female subjects were selected randomly depending on the availability and consent.
- **Collection of Blood Samples:** Blood samples will be collected by venepuncture using either the antecubital vein or the dorsal vein and dispensed into dipotassium EDTA anticoagulant bottles. All haematological parameters will be carried out by automatic methods.
- **Preparation of Serum Sample**: After collection of the whole blood, it will be allowed to clot by leaving it undisturbed at room temperature. This usually takes 15–30 minutes. The clot will be removed by centrifuging at 1,000–2,000 ×g for10minutes in a refrigerated centrifuge.
- **Biochemical Examination**
    - Serum Iron Estimation: Serum iron estimation was done with the help of the Gen X Iron Estimation Kit manufactured by the Proton Biologicals India Pvt. Ltd.
    - Total Iron Binding Capacity (TIBC): TIBC estimation was done with the help of TIBC Direct Colorimetric Kit manufactured by the Proton Biologicals India Pvt. Ltd.
    - Serum Ferritin Assay: Serum ferritin assay will be done with the help of Proton GenX Ferritin Test Kit manufactured by the Proton Biologicals India Pvt. Ltd.
- **Hematological Examination**
    - Automated Blood Count (Complete Blood Count): A complete blood count (CBC), also known as full blood count (FBC), will be analyzed by the haematology analyser (model – Cellenium-21) used for the in vitro diagnostic testing of whole blood specimens. This instrument has many different components to analyze different elements in the blood. The cell counting component counts the numbers and types of different cells within the blood. The results are printed out or sent to a computer for review HCT Measurement. The height of the impulse generated by the passage of a cell through the

microaperture is directly proportional to the volume of the analyzed RBC. The haematocrit is measured as a function of the numeric integration of the MCV. Complete Blood Count tests are –

1. Haemoglobin (Hb) concentration.
2. Examination of blood film (smear) for, - Assessment of red cell morphology

- **StatisticalAnalysis**: Mean SD and ANOVA test will be done by Microsoft excel to find any significant difference in hematological and biochemical parameters of female subjects with IDA of different age group's <0.05 will considered as significant.

RESULTS

Results

The diagnosis of iron deficiency anaemia was evaluated with the three biochemical parameters *viz*. serum iron, serum ferritin, and percentage saturation of transferrin. Using these parameters it was found that 40 out of 45 patients were suspected to have iron deficiency anaemia (IDA) (Fig 1).

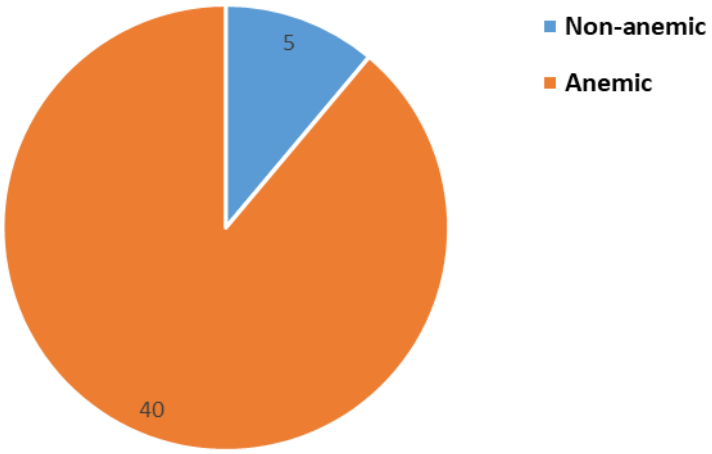


Fig 1: Representation of anemic and non-anemic subjects.

Age Distribution of IDA

Forty female subjects of IDA were grouped into 3 categories for further analysis based on reproductive age group. Among them the first group constituted of adolescent women of 15–25 years and 13 subjects were found in this group. The second group comprised of 14 women in the age group of 25–35 years which is focused on pregnancy phase. Lastly, the third group belonging to the age group of 35–45 years that represented nursing mothers in post pregnancy period.

Biochemical Parameters

The mean values of serum iron in first, second and third groups with IDA were 18 gm/dL, 20.81 gm/dL, 26.54 gm/dL respectively (Fig. 2). The mean total iron binding capacity (TIBC) was highest in first group with IDA (421 gm/dL) than other two groups (Fig. 3). The mean percentage saturation of transferrin in first, second and third groups of IDA were found to be 5.73%, 7.89% and 18.51% respectively (Fig. 4). The mean serum ferritin in IDA was 9.94 ng/mL for first group, followed by 20.52 ng/mL,68.14 ng/mL for second and third group accordingly (Fig. 5)

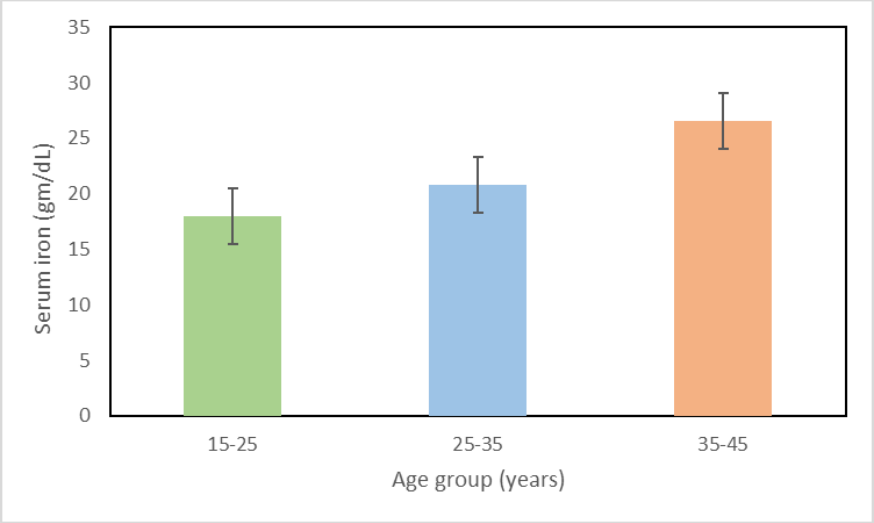


Fig 2. Distribution of serum iron in various age groups of subjects.

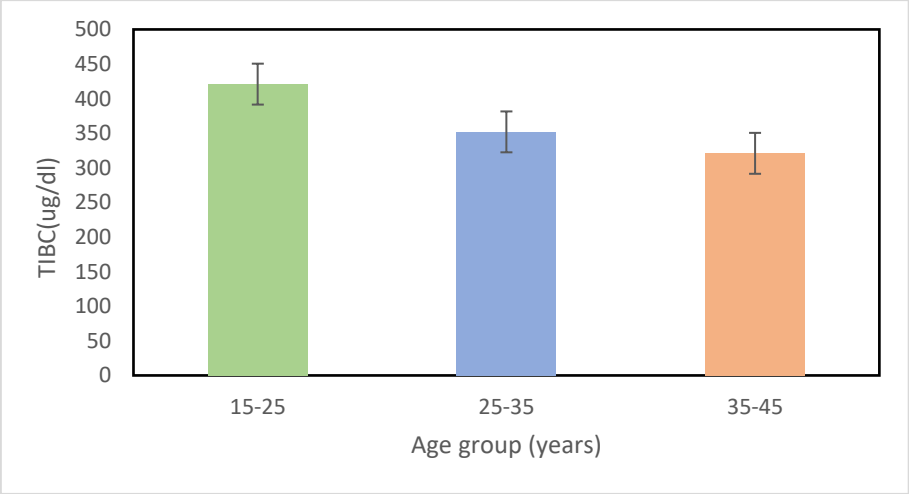


Fig 3. Distribution of TIBC in various age groups of subjects.

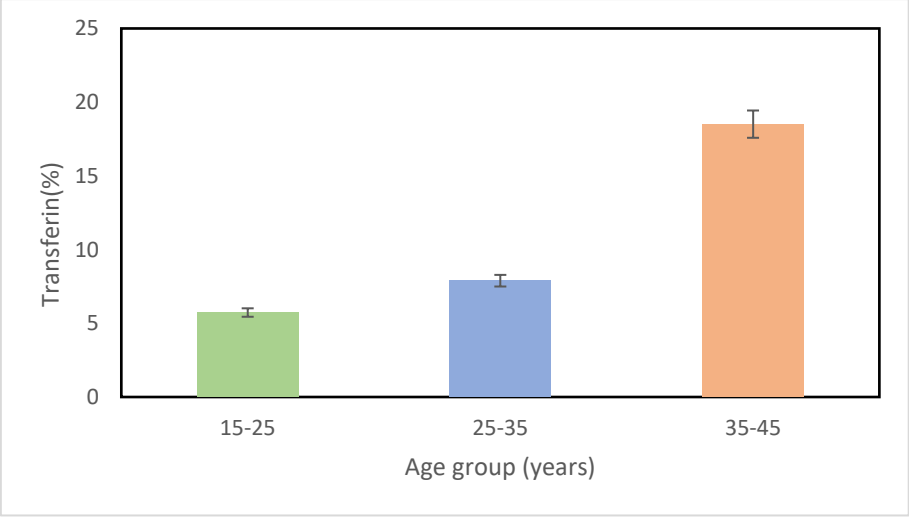


Fig 4. Distribution of saturation of transferrin percentage in different age groups of subjects.

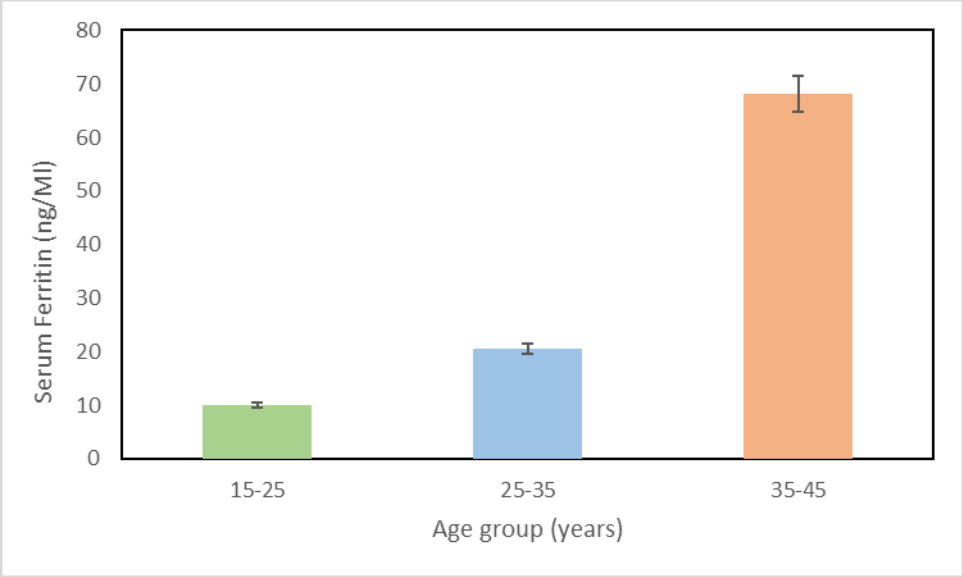


Fig 5. Distribution of serum ferritin among the three age groups.

Complete Blood Count

The blood samples were collected from suspected anemic patients. Then complete blood count was conducted for the following hematological values like hemoglobin (Hb), packed cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red cell distribution width (RDW). The results are represented graphically (Fig 6,7,8,9,10).

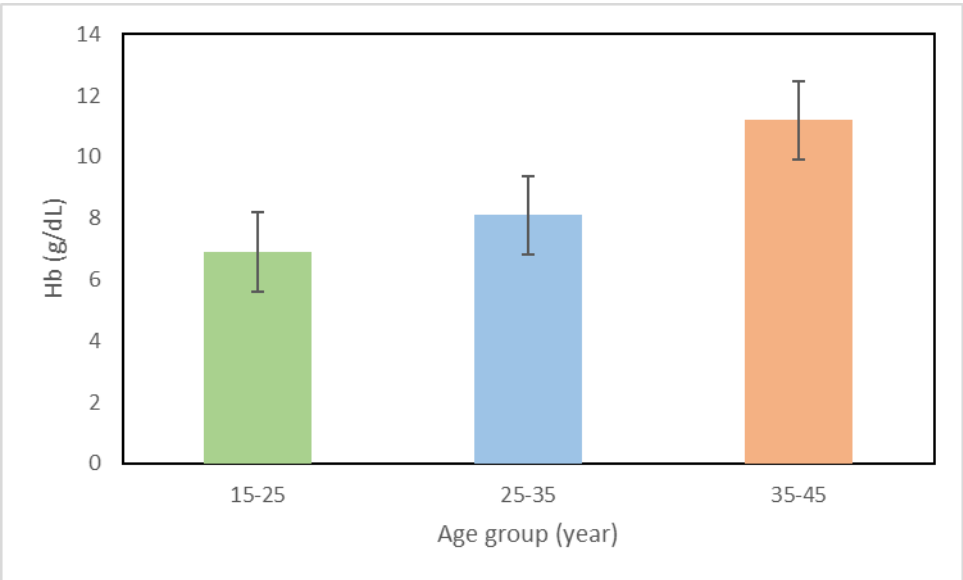


Fig 6. Distribution of mean value of Hemoglobin (gm/dL) among the three age groups.

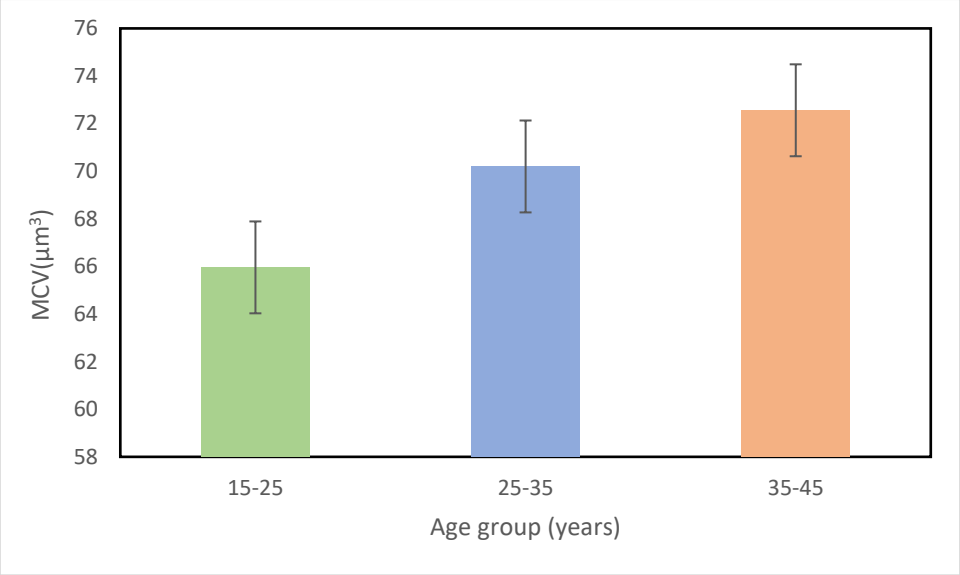


Fig 7. Distribution of mean corpuscular volume in various age groups of subjects.

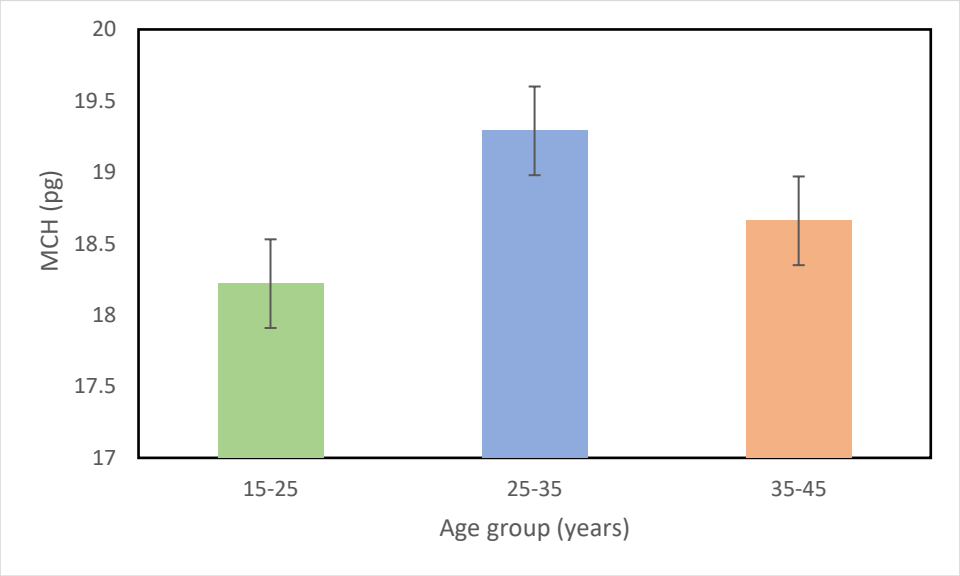


Fig 8. Distribution of mean value of mean corpuscular Hb (gm/dL) in the three age groups.

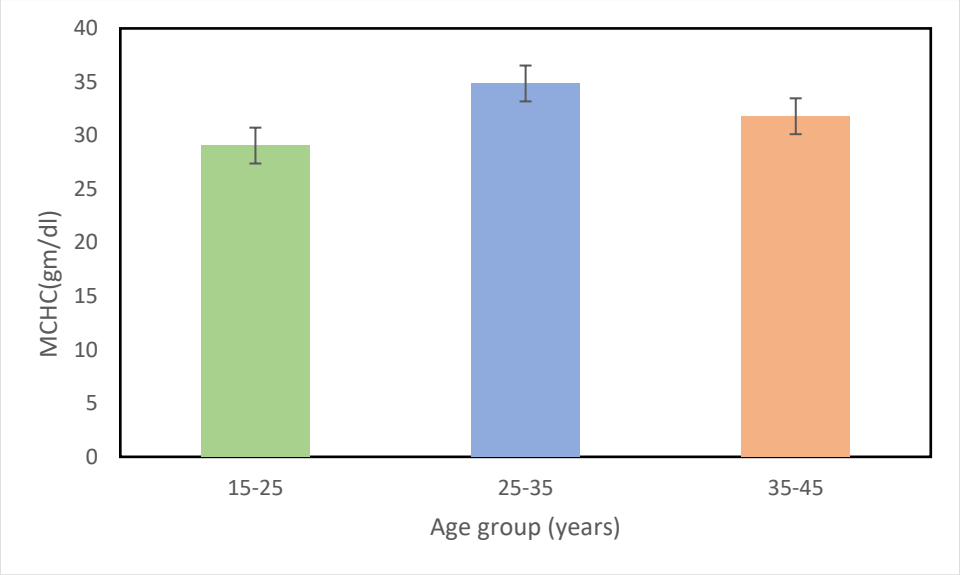


Fig 9. Distribution of mean value of mean corpuscular Hb concentration (gm/dL) among the three age groups.

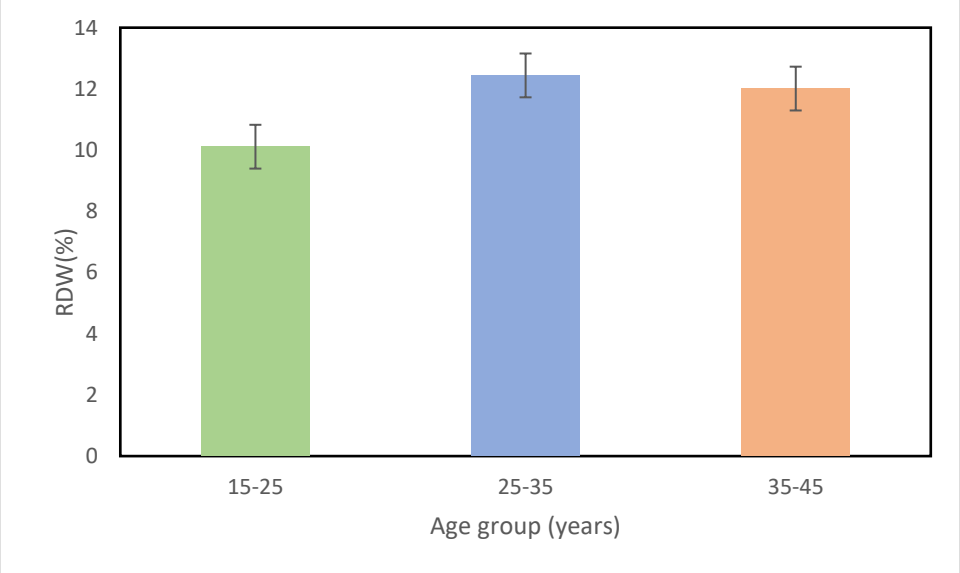


Fig 10. Distribution of mean value of Red cell distribution width percentage among the three age groups.

Statistical analysis

We conducted complete blood count for various hematological values like hemoglobin (Hb), packed cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red cell distribution width (RDW). One-way ANOVA test were used for comparison of hematological parameters. Results were considered to be statistically significant when the two-sided *P* value was less than 0.05 or ($P < 0.05$).

DISCUSSION

Discussion

In our study out of 40 female IDA subjects 32.5% were in age group between 15 and 25 years, 35% in age group between 25 and 35 years, 32.5% in age group between 35 and 45 years. It should be noted that iron supplements and increased iron stores have recently been linked to maternal complications (Scholl 2005). In contrast, our study showed that IDA is most commonly prevalent in the 15-25 year age group. These observations were taken from subjects of Taldangra block of Bankura district, West Bengal. It was observed that economically deprived and ignorant people are mostly affected. In clinical manifestations, weight loss, decrease of appetite, diarrhea, attention problems, and weakness were the most common complaints of the subjects with IDA. Also, it is equally common among the anaemic individuals who were not iron deficient. These observations are in accordance with the previous reports (Elwood et al., 1969). In our observation, the average MCV was found to be lowest in puberty and adolescent age group (65.96 μm^3) whereas the second and third group of women had mean MCV of 70.2 $\mu m^3$and 72.56 μm^3 respectively. Similarly the first group with 15-25 age suffered the most in IDA with least mean value of MCH was 18.22 pg, mean value of MCHC was 29.06%, and mean hemoglobin was 6.9 gm/dL. These observations are similar to the report (Bainton et al., 1971), which showed mean MCV to be 74 μm^3, mean MCH to be 20 pg, mean MCHC to be 28%, and mean hemoglobin to be 7.6 gm/dL in patients with IDA (Allen 1997).

In the present study, the mean value of serum iron in 15-25 age group of IDA was 18 gm/dL, which was significantly lower than second and third group (20.81 gm/dL and 26.54 gm/dL). In a study conducted in geriatric patients the mean was found to be 22.7 g/dL (Brittenham 2005) which is almost similar to our result. The principal limitation of the serum iron determination is variability in the values (Statland 1977), which may be due to both technical and physiologic factors (Dallman 1984) such as contamination of glassware and reagents with iron although the use of disposable, plastic equipment has reduced such contamination considerably. Among the biochemical tests, total iron binding capacity (TIBC) was increased in all groups of the IDA subjects viz. 421 μg/dL, 352 μg/dL, 321 μg/dL for first, second and third group respectively. The mean value of TIBC in these studied IDA subjects was higher than normal range of TIBC in non-anemic individuals. A study done in IDA patients the mean TIBC was found to be 413.6 μg/dL, which is also closer to our observation. Though increased value of TIBC indicates iron deficiency, the normal or even lower value may occur in iron deficiency anaemia (Lee 1996). So, TIBC was not included among the three biochemical parameters. Instead, in the present

study, we have used percentage saturation of transferrin, the values less than 16% occur in iron deficiency and anaemia of chronic diseases and values less than 5% are found only in iron deficiency (Rai et al., 1994). This trend was noticed also in our IDA study where 15-25 age group females were in more vulnerable state and low transferrin percentage in other two groups also indicated the iron deficiency.

The consequences of anaemia in women are enormous as the condition adversely affects both their productive and reproductive capabilities. Among women, iron deficiency prevalence is higher than among men due to menstrual iron losses and the extreme iron demands of a growing foetus during pregnancies, which are approximately two times the demands in the non-pregnant state. Worldwide, it is estimated that about 20 per cent of maternal deaths are caused by anaemia; in addition, anaemia contributes partly to 50 per cent of all maternal deaths (Galloway et al., 2002). First, anaemia reduces women's energy and capacity for work and can therefore threaten household food security and income. Second, severe anaemia in pregnancy impairs oxygen delivery to the foetus and interferes with normal intra-uterine growth, resulting in intra-uterine growth retardation, stillbirth, LBW and neonatal deaths. Therefore, anaemia is a major contributor to poor pregnancy and birth outcomes in developing countries as it predisposes to premature delivery, increased perinatal mortality and increased risk of death during delivery and postpartum.

SUMMARY & CONCLUSION

Summary & conclusion

Anaemia is a critical public health problem in India that affects women and children throughout the lifecycle. Women of reproductive age are at increased risk of anaemia because of chronic iron depletion during the menstrual cycle, inadequate dietary intakes and recurrent infections. Anaemia in girls during pregnancy is associated with premature births, low birth weight, and peri-natal and maternal mortality. Health and nutritional status during this phase are critical for the physical maturity, which in turn influence the health of the future offsprings.

Given the intensity of the problem in the country, intermittent IFA supplementation to all menstruating women would be a cost-effective strategy to build up iron stores and prevent anaemia. The following intervention is proposed for them:

- IFA supplementation (100 mg elemental iron and 500 mcg of folic acid) throughout the calendar year, i.e., 52 weeks, each year.

- Albendazole (400 mg) tablets for biannual de-worming for helminthic control.

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27/7/23

Examined

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