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Original Research Paper

Medicine

A PROSPECTIVE STUDY OF RED CELL INDICES FOR THE DIAGNOSIS OF EARLY NUTRITIONAL ANAEMIA IN NORMAL RANGE HAEMOGLOBIN CONCENTRATION

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ABSTRACT

Background: Anaemia is a condition where there is a decrease in total number of RBC or decrease in haemoglobin concentration.(1) According to the Oxford University, in 2019, the global prevalence of Anaemia in all ages was 22.8% (95% CI: 22.6-23.1) (2). With this magnitude of disease burden, It is important to diagnose anaemia before its full blown symptoms appear. This study has been done to emphasize on Red Cell Indices as a useful tool to diagnose anaemia in a patient who still fall under normal range of Haemoglobin.

Method: It's a prospective observational study in which 100 Heathy Adults were observed for a period of 6 months. Their basic demographic data has been collected, and they were subjected to routine haematology and Biochemistry investigations. Appropriate therapy was given to them and follow-up was done after 2 months and their respective data was compared.

Results: Out of 100 Adults, 75% have Low Normal Haemoglobin level. After intervention there was a significant difference between mean Pre-treatment and mean Post-treatment Haemoglobin concentration (14.176 to 14.943), Red Blood Cell Count (4.696 to 5.272), MCV of macrocytic Anaemia (108.99 to 89.32), MCV of dimorphic Anaemia (89.80 to 83.21), and MCH (30.24 to 28.63).

Conclusion: In our study, we noticed that even if an individual's Haemoglobin fall under normal range, he/she might be in Pre-Anaemic state where his/her Red-cell Indices will help us to early detect and treat the individual in order to decrease the morbidity and incidence of anaemia.

KEYWORDS :

INTRODUCTION

'Anaemia' a disease whose historical references can be traced even in 3rd/2nd BC in Charaka Samhita is still a disease with upcoming new discoveries, revealing and unwinding its different aspects. The first recommendation criteria for the diagnosis of anaemia were given by WHO in 1958 (world health organisation technical report series) on iron deficiency anaemia which from now is 64 years ago and based on few data and method that were inadequate range of normal as per WHO were lower limit of normal haemoglobin in male and female is 13g/dl and 12g/dl respectively.[1]

Anaemia is a late indicator of iron deficiency, so it is estimated that the prevalence of iron deficiency is 2.5 times that of anaemia. But what if we treat iron deficiency even before the patient anaemia is diagnosed so as to prevent the adverse clinical outcome of the anaemia.

The purpose of this study is to diagnose anaemia in preclinical early stage (subclinical stage) using various Red cell indices before it fulfils the diagnostic criteria, as the WHO criteria and early nutritional supplementation of replacement therapy so as to prevent the development of full blown anaemia and its consequences.

This has stimulated us to study the early diagnosis and treatment of nutrition anaemia, even in normal range haemoglobin concentration. Therefore with the hypothesis that even if we start replacement therapy in nutritionally deficient person having abnomal RBC indices, favouring either microcytic or macrocytic anemia and if haemoglobin concentration increases to higher level compared to the base line haemoglobin concentration then it supports our hypothesis of early replacement of nutritional supplement.

AIMS AND OBJECTIVES

- To Early Diagnose the Nutritional Anaemia at its subclinical stage with Study of RBC indices in Normal Range Haemoglobin concentration in healthy individuals.
- To correlate the indices with available Serum Iron, Serum Vitamin B12, in blood.
- Abnormal Indices will be treated by Vitamin B12, Iron, Folic Acid and to see the Improvement after 2 months of treatment with Improvement in Red Cell Indices
- Early Diagnosis of Anaemia in Pre-anaemic stage & Early treatment.

MATERIAL AND METHODS

The present prospective observational study entitled "A Prospective Study Of Red Cell Indices For The Diagnosis Of Early Nutritional Anaemia In Normal Range Haemoglobin Concentration" was conducted in the Department of Medicine, MGM Medical College and M.Y. Hospital, Indore (M.P.). The study title and other parameters were discussed in department of medicine and were approved, the project was projected before scientific committee with valuable suggestions and approval followed by approval from ethical committee. There is no objection from ethical point of view since there is no any new mediation apart from the standard guidelines it only deals with correction of anaemia by nutrition supplementation.

Duration of Study: 6 months

Sample Size:
A minimum 100 person attending Medicine Out Patient Department (OPD) were randomly selected in the Age group 18-60 years, coming for routine health check-up / fitness for jobs / coming with reports of executive check-up reports were followed up duration of 2 months.

Patient Selection:
Inclusion criteria
- All normal healthy individuals.
- All age group between 18 to 60 years.

Exclusion Criteria:
- All subjects symptomatic or with any known disease.
- All subject taking any drugs in any form or medical nutritional supplements in any form.
- Deranged LFT, RFT, Stool R/M, urine R/M.
- Subject refuses to give consent.

METHODOLOGY:
- Subjects of study included patients attending Medicine OPD.
- Informed consent was taken from all subject included in the study.
- All subjects in study had undergone a detailed history taking general examination and laboratory investigation.
- Records were maintained.
- Patient's identity was kept confidential.
- Randomly selected Healthy subjects were recruited Detailed history was taken. And all subjects was included if they were symptoms free and with no any sign of any disease. All subject were excluded if they have any disease, new trauma or symptomatic, or in age group of <18 years or > 60 years.
- Blood samples were taken as per patient consent and patient information documents and were sent for analysis for routine haematology (Haemoglobin, RBC, MCV, MCHC, MCH) and biochemistry (serum vitamin B12, serum iron). Routine biochemistry like FBS, 2 hr PPBS, Lipid Profile Renal Profile of all enrolled participants were analysed.
- After receiving the report, the subjects were enrolled as per study with their consent and was explained about the study and also explained about therapy to be given in form of nutritional supplements or therapeutic dose or in form of diet according to protocols. Subject with macrocytic type (MCV > 98f1, vitamin 12 < 180) of anaemia were given injection vitamin B12 1000 ug daily for 7 days followed by 1000 ug vitamin B12 injection once a month intramuscularly for two months along with tablet 10 μg methyl cobalamine with folic acid 5 mg once daily for two months along advice to take vitamin B12 rich diet ex. milk and milk products with non vegetarian diet. And Subject with microcytic type of anaemia with (MCV < 82 fl and serum iron < 50μg/dl) were given tablet ferrous sulphate 325 mg (60 mg of elemental iron) thrice a day along with food for two months and were advice to iron rich diet eg. green leafy vegetables and red meat.
- On follow-up subject were reviewed after 2 months with new CBC showing Haemoglobin, RBC indices (MCV, MCHC, MCH). Haematocrit. Platelet. and RBC count with 3 part auto analyser at central laboratory at our institute and were again asked for clinical examination and improvement if any was noted and again clinical proforma was filled-up.
- The data were collected and arranged in tabulated form and statistical analysis was done in form of tables, bar diagram and pie chart.

OBSERVATION AND RESULTS

Table 1: Age wise distribution of cases

Age Group (years)	No. of cases	Percentage
18-20	15	15.0
21-30	29	29.0
31-40	23	23.0
41-50	23	23.0
51-60	10	10.0
Total	100	100.0

Table 1 depicts the age wise distribution of the study population

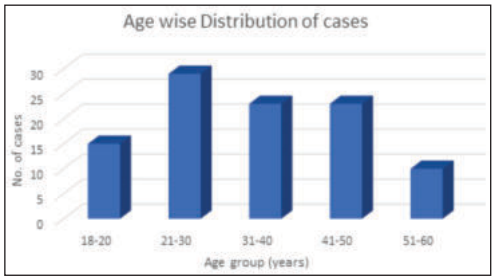

Figure 1 Age wise distribution of the study population

Table 2 : Sex wise distribution of cases

Sex	No. of Cases	Percentage
Male	64	64%
Female	36	36%
Others	0	0
Total	100	100%

Table 2 depict the Male-Female ratio of our study population

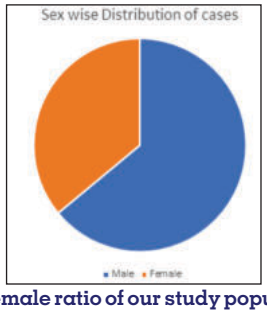

Figure 2 Male-Female ratio of our study population

Table 3 : Age and Sexwise distribution of cases

Age Group (years)	Male		Female	
	Numbers	Percentage	Numbers	Percentage
18-20	11	17.18	4	11.76
21-30	16	25.00	13	38.24
31-40	13	20.31	10	29.41
41-50	16	25.00	7	20.59
51-60	8	12.5	2	5.88
Total	64	100.0	34	100.0

Table 3 depict the age and sex wise distribution of cases in number and percentage

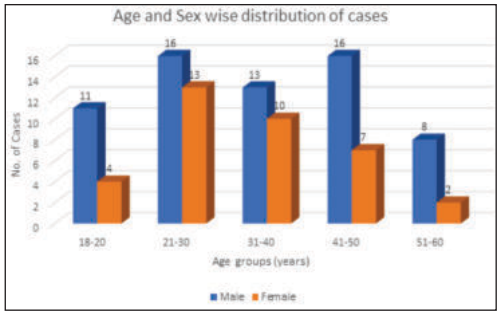

Figure 3 Age and sex wise distribution of cases in number and percentage

Table 4: Type of Anaemia

Type of Anaemia	No. of Cases	Percentage
Dimorphic	18	18.0%
Macrocytic	73	73.0%

Microcytic	9	9.0%
Total	100	100.0%

Table 4 depict the type anaemia in number of cases and their percentage

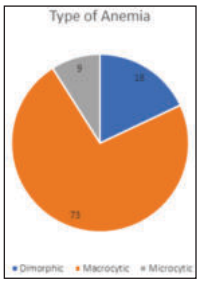

Figure 4:Type Anaemia In Number Of Cases And Their Percentage

Table 5: Pre-treatment according to their Sex

Sex	Low Normal Haemoglobin		High Normal Haemoglobin	
	Number	Percentage	Number	Percentage
Male	61	81%	3	12%
Female	14	19%	22	88%
Total	75	100%	25	100%

Table 5 depicts Pre-treatment Male Female total population distribution and their percentage according to low normal haemoglobin and high normal haemoglobin concentration.

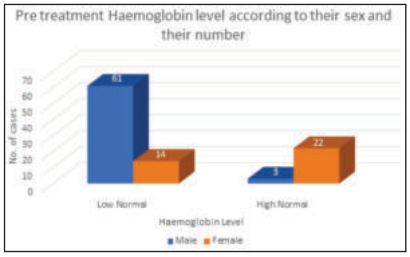

Figure 5 Pre-treatment Male Female total population distribution and their percentage according to low normal haemoglobin and high normal haemoglobin concentration

Table 6: Post Treatment according to their sex

Sex	Low Normal Haemoglobin		High Normal Haemoglobin	
	Number	Percentage	Number	Percentage
Male	52	88%	12	29%
Female	7	12%	29	71%
Total	59	100%	41	100%

Table 6 depicts Post treatment Male Female total population distribution and their percentage according to low normal haemoglobin and high normal haemoglobin

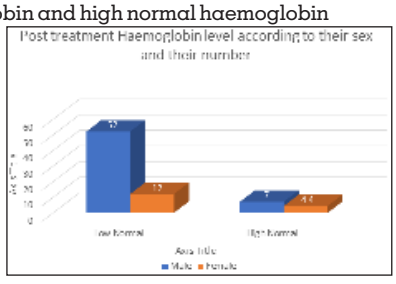

Figure 6 Post treatment Male Female total population distribution and their percentage according to low normal haemoglobin and high normal haemoglobin

Table 7: Comparison of haemoglobin before and after treatment

Study Parameter	Mean	Standard Deviation	P-value
Pre- treatment Haemoglobin level	14.176	1.129	0.000
Post treatment Haemoglobin level	14.943	1.081	

*Highly significant

The **paired 't'** test was used for finding out the difference between the pre and post treatment. The mean Haemoglobin level before treatment was obtained as 14.176 ±11.129 and the mean post Haemoglobin after treatment was obtained as 14.943±1.081. The degree of freedom was 99, with a 't' value of 19.07 and p value obtained was 0.000, which is highly significant. Thus, there was a high significant difference between the Haemoglobin level before treatment and the Haemoglobin level after treatment, showing the effectiveness of the intervention used.

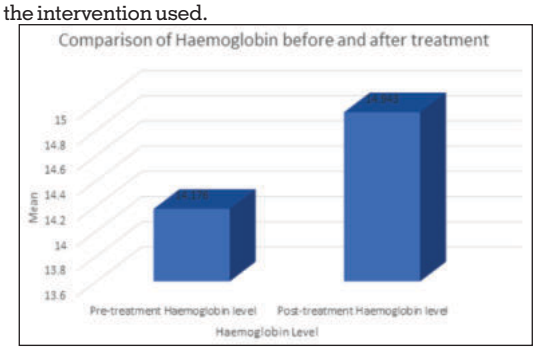

Figure 7: Comparison of haemoglobin before and after treatment

Table 8: Comparison of Red blood cell count before and after treatment.

Study Parameter	Mean	Standard deviation	P value
Pre-treatment Red Blood Cell count	4.696	0.574	0.00
Post-treatment Red Blood cell count	5.272	0.406	

* Highly significant

The paired 't' test was used for finding out the difference between the pre and post treatment. The mean RBC count before treatment was obtained as 4.696±.574 and the mean RBC count after treatment was obtained as 5.272±.406. The degree of freedom was 99, with a t value of -14.37 and p value obtained was 0.000, which is highly significant. Thus, there was a highly significant difference between the RBC count before treatment and the RBC count after treatment, showing the effectiveness of the intervention used.

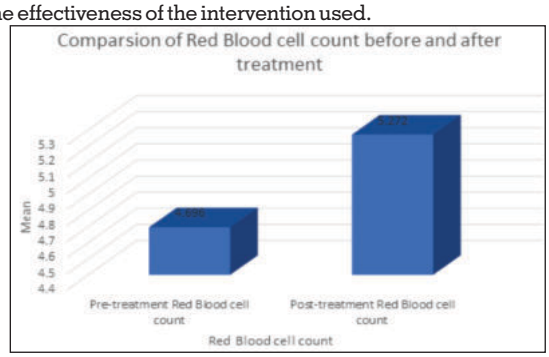

Figure 8: Comparison of Red blood cell count before and after treatment.

Table 9: Comparison of MCV of macrocytic anaemia before and after Treatment

Study Parameter	Mean	Standard Deviation	P- value
Pre-treatment MCV	108.99	19.03	0.000
Post-treatment MCV	89.32	7.51	

* Highly significant

The paired 't test was used for finding out the difference between the pre and post treatment. The mean MCV before treatment was obtained as 108.99±19.03 and the mean MCV after treatment was obtained as 89.324±7.51. The degree of freedom was 72, with a t value of 11.115 and p value obtained was 0.000, which is highly significant. Thus, there was a highly significant difference between the MCV of macrocytic anemia before treatment and the MCV of macrocytic anemia after treatment, showing the effectiveness of the intervention used.

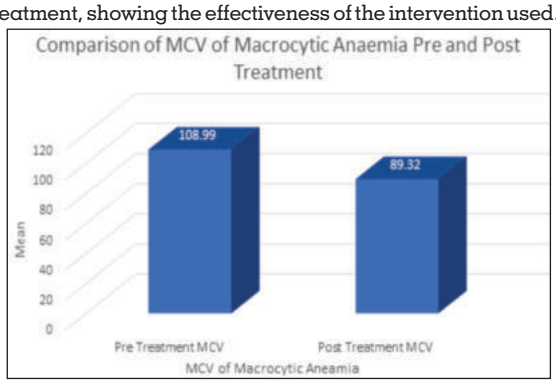

Figure 9: The bar diagram depicts mean pre-treatment MCV and mean post treatment MCV in macrocytic anaemia indicating fall in post treatment MCV level toward normal range MCV.

Table 10: Comparison of MCV of microcytic anaemia before and after treatment.

Study Parameter	Mean	Standard deviation	P value
Pre-treatment MCV	73.65	9.73	0.028
Post-treatment MCV	84.08	4.26	

* Not significant

The paired 't' test was used for finding out the difference between the pre and post treatment. The mean MCV before treatment was obtained as 73.65±9.73 and the mean MCV after treatment was obtained as 84.08±4.26. The degree of freedom was 8, with a 't' value of -2.686 and p value obtained was 0.028, which is not significant. Thus, there was no significant difference between the MCV of microcytic anemia before treatment and the MCV of microcytic anaemia after treatment. It may be due to small sample size.

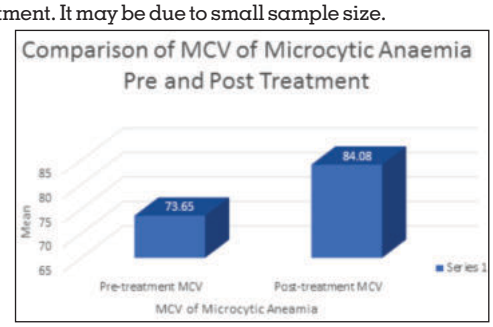

Figure 10: The bar diagram depicts mean pre-treatment MCV and mean post treatment MCV level indicating rise in post treatment MCV level toward normal range of MCV.

Table 11: Comparison of MCV of Dimorphic anaemia before and after treatment

Study Parameter	Mean	Standard Deviation	P value
Pre-treatment MCV	89.80	10.73	0.003
Post-treatment MCV	83.21	6.82	

* Significant

The paired 't' test was used for finding out the difference between the pre and post treatment. The mean MCV before treatment was obtained as 89.84±11.06 and the mean MCV after treatment was obtained as 83.28±7.02. The degree of freedom was 17, with a 't' value of 3.499 and p value obtained was 0.003, which is significant. Thus, there was significant difference between the MCV of dimorphic anaemia before treatment and the MCV of dimorphic anaemia after treatment, showing the effectiveness of the intervention used.

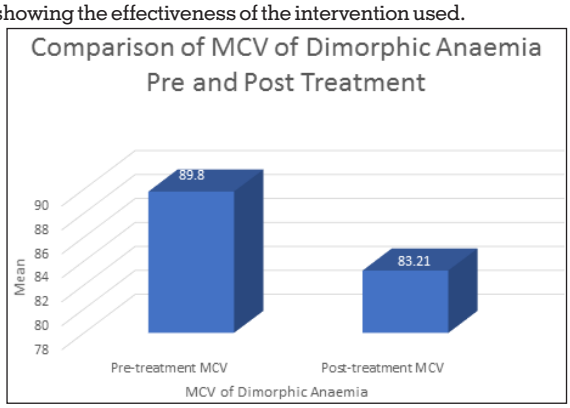

Figure 11: The bar diagram depicts mean pre-treatment and mean post treatment MCV level in anaemia indicating fall in MCV level.

Table 12: Comparison of MCH before and after treatment

Study Parameter	Mean	Standard deviation	P value
Pre-treatment MCH	30.24	3.71	0.000
Post-treatment MCH	28.63	2.55	

* Highly significant

The paired 't' test was used for finding out the difference between the pre and post treatment. The mean MCH before treatment was obtained as 30.24±3.71 and the mean MCH after treatment was obtained as 28.63±2.55. The degree of freedom was 99, with a 't' value of 5.222 and p value obtained was 0.000, which is highly significant. Thus, there was a highly significant difference between the MCH before treatment and the MCH after treatment, showing the effectiveness of the intervention used.

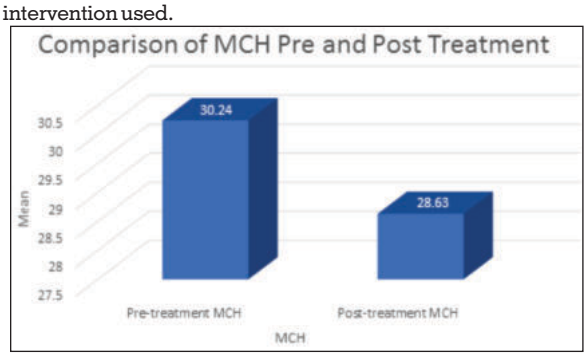

Figure 12: The bar diagram depicts mean Pre-treatment and mean Post-treatment MCH.

Table 13: Comparison of MCHC before and after treatment

Study Parameter	Mean	Standard Deviation	P-value
Pre-treatment MCHC	31.98	19.88	0.923
Post-treatment MCHC	32.18	3.31	

* Non-significant

The paired 't' test was used for finding out the difference between the pre and post treatment. The mean MCHC before treatment was obtained as 31.98±19.88 and the mean MCHC after treatment was obtained as 32.18±3.31. The degree of freedom was 99, with a 't' value of -.097 and p value obtained was 923, which is not significant. Thus, there was a non-significant difference between the MCHC before treatment and the MCHC after treatment, showing the no effectiveness of the intervention used.

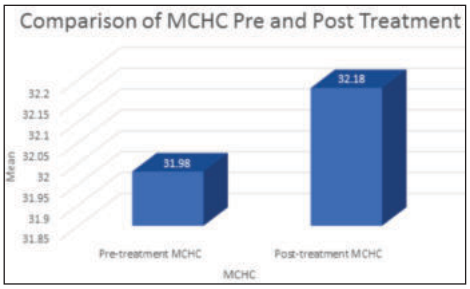

Figure 13 : The bar diagram depicts mean pre-treatment MCHC and post treatment MCHC indicating rise in rise of MCHC post treatment.

DISCUSSION

After involving the subject for study, they were divided into iron deficiency as microcytic and vitamin B12, folic acid deficiency as macrocytic groups and were given iron supplements and vitamin B12, folic acid intra muscular injections respectively. Those having both deficiency of iron and vitamin B12 and having dimorphic picture were given both supplementation and were followed after 2 month. It was found there was significant improvement in haemoglobin concentration. The mean Haemoglobin level before treatment was obtained as 14.176±1.129 and the mean Haemoglobin after treatment was obtained as 14.943±1.081. The p value obtained was 0.000, which is highly significant. Thus, there was a highly significant difference between the Haemoglobin level before treatment and the Haemoglobin level after treatment, showing the effectiveness of the intervention used. Similarly for RBC indices the mean MCV of macrocytic anaemia before treatment was obtained as 108.99±19.03 and the mean MCV after treatment was obtained as 89.32±7.51. The p value obtained was 0.000, which is highly significant. Similarly for RBC indices the mean MCV of microcytic anaemia before treatment was obtained as 73.65±7.73 and the mean MCV after treatment was obtained as 84.08±4.26. The p value obtained was 0.028, which is not significant. It may be due to small sample size.

The mean MCH before treatment was obtained as 30.24±3.71 and the mean MCH after treatment was obtained as 28.63±2.55. The p value obtained was 0.000, which is highly significant. The mean MCHC before treatment was obtained as 31.98±19.88 and the mean MCHC after treatment was obtained as 32.18±3.31. The p value obtained was 0.923, which is not significant. In our study there is definite improvement in haemoglobin concentration and normalization of RBC indices as well as subjective improvement of wellbeing with nutritional supplement, dietary advise and therapeutic intervention in low normal haemoglobin concentration.

The first recommended criteria for iron deficiency Anaemia and cut-offs were defined as mild, moderate and severe Anaemia for particular age and sex in 1958 by WHO[1] which are still vogue now.

Haemoglobin levels to diagnose Anaemia at sea level (g/100 ml)

Adult Males :	13
Adult Females:	12

The recommendation was given when there was least amount of digitalisation and data collection, comparison as compared to current scenario of data collection and analysing, leading to short coming in studies which are in general and not individualised [3] for lower limit of normal haemoglobin concentration. There are various other definitions from various other sources which is provided in the table below, which makes the diagnosis of Anaemia with a clear cut-off value of Haemoglobin even a more cumbersome process.

Source	Men (gm/dL)	Women (gm/dL)	Percent normal below cut-off	Effect of race
WHO (Blanc et al) [(1)]	13	12	Not provided	Not provided
Jandl[(3)]	14.2	12.2	2.5	Discussed
William (Beutler et al)	14.0	12.3	2.5	Not provided
Wintrobe (Lee et al)	13.2	11.6	Not provided	Not provided
Rapaport	14	12	Not provided	Not provided
Goyette	13.2	11.7	5	Black's haemoglobin 0.5 g/dL lower
Tietz	13.2	11.7	Not provided	Not provided
Hoffman et al	13.5	12.0	2.5	Not provided

So this study was done in keeping view of changes of RBC indices with variation of haemoglobin concentration and brought us a New Preclinical stage; PRE-ANEMIA which is defined as A stage in Nutritional Deficiency Anaemia when there is presence of Abnormal Red Cell Indices but Haemoglobin concentration of the individual comes under normal range as per WHO criteria.

So, this study is beneficial in current knowledge of early diagnosis by interpreting and giving weightage to RBC indices and supplementation of nutritional deficiency leading to anaemia in future, in normal range haemoglobin population and thereby decreasing the future burden of anaemia in population.

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