

Morphological and biochemical profile of anaemia- Does it help a step further in under diagnose cases in a hospital based study

Venkat Raghavan A.T.M¹, Vaishali D. Kotasthane^{2*}, Dhananjay S. Kotasthane³

¹Post Graduate, ²Associate Professor, ³Professor and HOD, Dept. of Pathology, Mahatma Gandhi Medical College and Research Institute, Pondicherry, India

***Corresponding Author:**

Email: vaishalikotasthane@gmail.com

Abstract

Introduction: Anaemia is one of the preventable disease burden worldwide, especially in developing nations like India. There are varied aetiologies for anaemia; Iron deficiency being the most common cause. Age, sex, geographical and comorbid factors play important role in the morphological patterns of anaemia. Anaemia, if undetected can cause morbidity and mortality which can be prevented.

Aims and objectives: To identify morphological patterns and severity of anaemia in patients of all age groups and correlate the findings of automatic hemoanalyser, peripheral smear and biochemical parameters like iron studies, vitamin B 12 and folic acid; to correlate these findings with age, sex, morphological type and to find out the commonest pattern.

Materials and Methods: This cross sectional study comprised of 300 anaemic patients over a period from December 2015 to July 2017 in Mahatma Gandhi Medical College and Research Institute, Pondicherry.

Results: In our study of 300 cases, microcytic hypochromic anaemia was the commonest anaemia in all age groups (66%), followed by dimorphic anaemia (23%). Also, prevalence of microcytic hypochromic anaemia was highest in our study (p value<0.005) as compared to other studies. Two (1.01%) of 197 microcytic hypochromic cases showed elevated levels of Iron ferritin and increased saturation and decreased TIBC suggesting thalassemia trait. Remaining 195 microcytic hypochromic anaemic cases showed reduced levels of serum Iron, ferritin and increased levels of TIBC. Amongst 23% dimorphic anaemia, 76.4% patients showed reduced levels of serum Iron and ferritin with increased TIBC, 7.3% showed reduced levels of vitamin B 12, 10.2% showed reduced folate levels. Among 8% patients with Macrocytic anaemia, 80% patients had both vitamin B12 and folate deficiency, 12% had folate deficiency alone, 8% patients showed normal vitamin B 12 and folate levels. Normocytic normochromic anaemia accounted to 3%, of which 5 cases showed reticulocytosis, indicating hemolytic anaemia. Regarding age, majority were adults, in which female predominance was seen (M:F ratio is 1:1.8), majority (68.3%) showed severe degree of anaemia.

Conclusion: In this hospital study, microcytic hypochromic anaemia was the most common morphological type of anaemia in which two asymptomatic cases of presumptive Thalassemia trait detected due to correlation between complete blood count, peripheral smear study and further biochemical studies. These correlations proved to be definitive in establishing diagnosis of aetiology of anaemia. This inclusive approach towards conclusive diagnosis of anaemia will help to lessen the burden associated with it.

Keywords: Anaemia, Blood Indices, Microcytic hypochromic, Dimorphic, Normochromic normocytic, Iron studies, Serum B12 levels, Serum folate levels.

Introduction

Anaemia, though a preventable and curable condition, it poses a great threat to the health of the individual at different age groups. Nearly, two billion people in the world are believed to be anaemic.¹ Anaemia affects all the age groups, from neonates, children, adult men and women, particularly pregnant women. The prevalence of anaemia in developing countries is 39% in children <5 years, 48% in children 5- 14 years, 42% in women 15-59 years, 30% in men 15-59 years, and 45% in adults >60years.¹ Anaemia can lead to physical emancipation in individuals.² Though paediatric population and females are more susceptible adult males are also affected due to inadequate food intake or gastrointestinal malignancy.

To establish the diagnosis of anaemia, hemoglobin, hematocrit or total red blood cell count are used. At times, these alone are not sufficient to diagnose, but a morphological typing of anaemia on peripheral smear is necessary to complete the typing of anaemia. If these

findings are supplemented by further studies like iron, B12 and folic acid study, it helps the clinician to determine the exact cause of anaemia and accordingly its management.

Many studies are done targeting a limited age group of individuals; very few studies are carried out on the individuals of different age groups and few studies established a geographical and epidemiological reason behind the prevalence of particular type of anaemia.³ Also, very few studies have done further biochemical tests i.e iron studies and Serum B12, folate levels to confirm the diagnosis after morphological typing of anaemia.

Therefore, the aim of present study was to know the morphological patterns of anaemia in adult male, female and paediatric patients in our tertiary care centre with the help of haemoglobin values, blood indices values on automatic hemoanalyser and to correlate the results with peripheral smear study and further biochemical studies like serum iron, ferritin, total iron

binding capacity, serum vitamin B12 and folic acid levels. This was the benefit in our study which included Biochemical studies which were uncommon in other Indian studies.

Materials and Methods

The present study was cross-sectional study which was conducted in Mahatma Gandhi Medical College and Research Institute (MGMCRI) in Pondicherry, a tertiary care centre after obtaining Institutes' Human Ethics Committee approval. The study period was from December 2015 to July 2017 done in the Department of Pathology, MGMCRI. Inclusion criteria of the study was all consecutive cases of anaemia based on low haemoglobin levels than the normal for that age and gender came during study period. Consent was obtained from patients who were fulfilling inclusion criteria of the study who were referred to central laboratory for investigation. Blood samples were collected by venepuncture method after proper aseptic precautions in EDTA vacutainer and were analysed on eight part automated haemato-analyser Horiba PENTRA DF for complete blood count(CBC),Haemoglobin levels, red cell indices (MCV, MCH, MCHC), Red Distribution Width and values were noted. Peripheral blood smear were stained with Leishman stain and relevant findings were noted. Supravital staining (New methylene blue) was performed for reticulocyte count wherever required. The further biochemical tests for serum ferritin, folic acid, vitamin B12 were performed accordingly on the principle of chemiluminescence method and serum iron by automated charting analyser after morphological typing of anaemia on peripheral smear. As serum iron studies were done, bone marrow aspiration was indicated in very few cases like pancytopenia on peripheral smear. Bone marrow smears were stained with Leishman's stain. These findings were also compared with clinical data like age and gender.

Anaemia was defined according to World Health Organization (WHO) as a hemoglobin level below 11g/dl in children from six months to eleven years, below 12 g/dl for twelve to fourteen years, below 12g/dl for non-pregnant women, and below 11 g/dl in pregnant women and less than 13 g/dl in men.¹ These were inclusion criteria for anaemia to include in the study. Also, grading of anaemia into mild, moderate and severe was done according to WHO. Morphological classification of anaemia was done based on MCV values and Peripheral smear morphology of RBCs. Microcytic anaemia was considered when Mean Corpuscular volume (MCV) was less than 80 fl and PS showing microcytic RBCs, for Normocytic anaemia-MCV 81-99 fl and normochromic normocytic morphology on PS, Macrocytic anaemia-MCV>100 fl and PS showing macrocytic normochromic RBCs.⁴

Statistical Methods: Percentage was used as a descriptive statistics to explain the distribution. Pie diagram and bar diagrams were used for simple data interpretation. For continuous variables, Parametric test like Chi-square was performed for the data which was following normal distribution, P value was calculated and p value <0.05 was considered significant.

Results

In the study period, 300 patients with anaemia were studied.

Age Distribution: Out of 300 anaemic patients,5(1.7%) cases were children aged from 6 to 59 months,3(1%) were 5 to 11 years of age, 3(1%) were from 12 to 14 years of age and 289 (96.3%)cases were adult patients above 15 years.

Gender Distribution: In the present study, majority of the anaemic patients were adult i.e above 15 years; out of which 64.4 % were females and 35.6 % were males. M:F ratio of the present study was 1:1.8.

Severity of Anaemia: In all the age groups, majority showed severe degree of anaemia. In total 300 cases, 12(4 %) patients had mild degree of anemia, 83(27.6%) had moderate degree and 205 (68.3%) had severe degree of anaemia. [Table 1]

Morphological patterns of Anaemia: In paediatric age groups, all patients showed Microcytic hypochromic anaemia. In adults, predominant anaemia was Microcytic Hypochromic anaemia accounting to 186 (66%) cases, followed by 68 (23%) patients with dimorphic anaemia, 25(8%) showed Macrocytic anaemia and 10 (3%) had Normocytic Normochromic anaemia. [Table 2]

Blood Indices, RDW and Iron Studies in Microcytic Hypochromic Anaemia

MCV, MCH and MCHC in Microcytic Hypochromic Anaemia: In 197 patients with microcytic anaemia on peripheral smear [Table 3], besides low haemoglobin concentration,Red cell indices like MCV also was decreased below 80 fl in 194 cases(98.4%), the other 3 (1.5%)cases showed MCV within normal range. Similarly,193 (97.9%) patients had MCH levels less than 27 pg,3(1.5%) patients had MCH within normal range(27 to 32 pg) and 1(0.5%) patient had MCH more than 32 pg.Regarding MCHC,126(63.9%) patients had MCHC less than 30 g/dl,60(30.4%) patients had MCHC within 30-35 g/dl and 11(5.5%) patients had MCHC more than 35 g/dl.

Red Cell Distribution Width (RDW): Red cell distribution width(RDW) was increased more than 14.5 % in 191(96.4%) cases of microcytic hypochromic anaemiaout of 197 total cases.4(2%) cases showed RDW within normal range of 9.0-14.5 %,2 (1%) patient had RDW less than 9.0 %.

Iron Studies in Microcytic hypochromic Anaemia: In 197 patients with microcytic hypochromic anaemiaIron studies like serum Iron, serum ferritin and TIBC were performed. Two adult cases (1%) with

reduced RDW showed elevated serum iron and ferritin level with saturation showing 70% pointing towards presumptive diagnosis of Thalassemia trait. In remaining 195 patients, serum Iron and ferritin were found to be decreased, TIBC was increased; thus, ruling out other differential diagnosis of hypochromic microcytic i.e thalassemia trait, sideroblastic anaemia and anaemia of chronic diseases and confirming the peripheral diagnosis of iron deficiency anaemia in adults.

Blood Indices, RDW, IRON, B12, Folic Acid Studies Indimorphic Anaemia

MCV, MCH and MCHC in Dimorphic Anaemia: Amongst 68 cases of dimorphic anaemia, 52 (76.4%) cases had MCV less than 80 fl and 16 (23.5%) had MCV within normal range [Table 4]. Similarly, 64 (94.1%) patients had MCH less than 27 pg and 4(5.8%) had MCH within normal range. No patient had MCH above 32 pg. In case of MCHC, 47(69.1%) had MCHC less than 30 g/dl and 21(30.8%) had MCHC within normal range. No patient had MCHC above 35 g/dl.

RDW in Dimorphic Anaemia: Out of 68 patients of dimorphic anaemia, 5(7.3%) of them had RDW within normal range and 63(92.6%) had RDW more than 14.5%. None of the patient had RDW less than 9.0%.

Iron Studies and Vitamin B12 and Folic Acid Studies: In 68 patients with dimorphic anaemia, 52(76.4%) showed reduced levels of serum Iron and ferritin with increased TIBC, 5(7.3%) patients showed reduced levels of vitamin B 12, 7 (10.2%) patients showed reduced folate levels and 4(5.8%) patients showed combined deficiency of Iron, vitamin B 12 and folic acid. [Table 4]

Blood Indices, RDW, B12, Folic Acid Studies Inmacrocytic Anemia

MCV, MCH and MCHC in Macrocytic Anaemia: Amongst the 25 cases of Macrocytic anaemia [Fig. 1], 23(92%) patients had elevated MCV above 100fl; remaining 2 (8%) patients had MCV less than 80 fl. Similarly, 8(32%) patients had MCH less than 27 pg, 10 (40%) patients had within normal range and 7(28%) patients had MCH more than 32 pg. In case of MCHC, 3(12%) patients had MCHC less than 30 g/dl and 22(88%) had MCHC within normal range. No patient had MCHC above 35 g/dl.

RDW in Macrocytic Anaemia: Out of 25 patients of Macrocytic anaemia, 19(76%) patients showed RDW

within normal range and 6(24%) patients had RDW above 14.5. None of the patients with Macrocytic anaemia had RDW less than 9.0.

Vitamin B12 and Folic Acid Studies in Macrocytic Anaemia: In the 25 patients with Macrocytic anemia, 20(80%) patients had vitamin B12 less than 125 pmol/l, which is significant to call Megaloblastic anaemia. In other 5(20%) patients, vitamin B12 was 125 pmol/l and above

Amongst 25 patients with Macrocytic anaemia, 23(92%) patients had reduced serum folate levels less than 3 ug/l, whereas remaining 2(8%) cases had serum folate levels 3ug/l and more than it.

Thus, 20(80%) patients showed combined deficiency of both vitamin B 12 and folic acid, 3(12%) patients showed reduced levels of vitamin B 12 alone and 2(8%) patients showed normal range of both vitamin B 12 and folic acid out of these 25 patients with Macrocytic anaemia.

Bone Marrow Studies in Macrocytic Anaemia: Out of 25 patients with Macrocytic anaemia, 5 patients with pancytopenia underwent Bone marrow aspiration which showed Erythroid hyperplasia with Megaloblastic maturation. [Fig. 2]

Blood indices, Rdwinnormocytic Normochromic Anemia

MCV, MCH and MCHC in Normocytic Normochromic Anaemia: In 10 patients who had Normocytic normochromic anaemia [Fig. 3], all had the MCV within normal range (80-100 fl). Similarly, 8(80%) patients had MCH less than 27 pg and 2(20%) patients had MCH within normal range. No patient had MCH more than 32 pg. In case of MCHC, 8(80%) patients had MCHC less than 30 g/dl and 2(20%) had MCHC within normal range. No patient had MCHC above 35 g/dl.

RDW in Normocytic Normochromic Anaemia: Out of 10 patients with Normocytic Normochromic anaemia, all of them had RDW within normal range

Reticulocyte Count: In all 10 cases of Normocytic normochromic anaemia, Reticulocyte count was done using supravitalstain (New methylene blue). Reticulocyte count was increased more than 2.5% among 5 cases and other 5 cases showed Reticulocyte count within normal limits. [Fig. 4]

Table 1: Age wise distribution of Degree of Anaemia (n=300)

Age group	Degree of Anaemia			Total N (%)
	Mild	Moderate	Severe	
6-59 months	0	02	03	5(1.7%)
5-11 years	0	01	02	3(1%)
12 -14 years	0	0	03	3(1%)
15 years and above	12	80	197	289(96.3%)
Total	12 (4%)	83(27.6%)	205(68.3%)	300(100%)

Table 2: Comparative study between MCV values and Biochemical studies in dimorphic anaemia [n=68]

Biochemical studies	MCV<80 fL 52(76.4%)	MCV 80 -100 fL 16(23.5%)	MCV>100 fL 0(0%)	Total 68 (100%)
Iron Deficiency (n=52)	52(76.4%)	0	0	52
B12 deficiency (n=5)	0	5(7.3%)	0	05
Folic Acid deficiency (n=7)	0	7 (10.2%)	0	07
Combined Iron,B12 & Folic acid deficiency (n=4)	0	4(5.8%)	0	04

Table 3: Degree of anaemia in various studies

S.No	Studies	Mild	Moderate	Severe
1	Ghalaut et al(2016)	25%	30%	45%
2	Nasrin A. Qureshi et al(2015)	46.34%	43.44%	10.22%
3	Gerardo Alvarez-Uria et al(2014)	30%	60%	10%
4	Present study	4%	27.6%	68.3%

Table 4: Morphology of anaemia in various studies

S. No	Studies	Microcytic hypochromic anaemia	Dimorphic anemia	Macrocytic anemia	Normocytic Normochromic anaemia
1	Banushree C Srinivasamurthy (2013)	44.5%	24%	4.5%	27%
2	Nasrin A. Qureshi et al (2015)	46.5%	1.36%	9.8%	42.25%
3	Sandeep Barve et al (2015)	45%	10%	14%	19%
4	Present study	66% ^{abc}	23%	8%	3% ^{abc}

Note: a – Comparison of present study with study of Banushree Srinivasamurthy, b-Comparison of present study with study of Nasrin A Qureshi et al and c-Comparison of present study with study of Sandeep Barve et al.

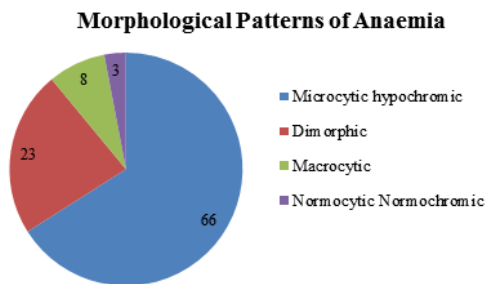


Fig. 1: Percentage distribution of morphological patterns of anaemia

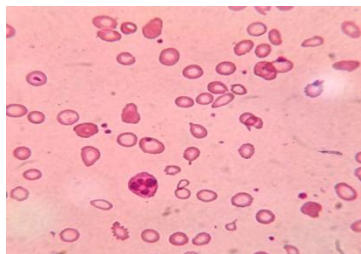


Fig. 2: Peripheral smear showing microcytic hypochromic anaemia with anisocytosis (Leishman 100 x)

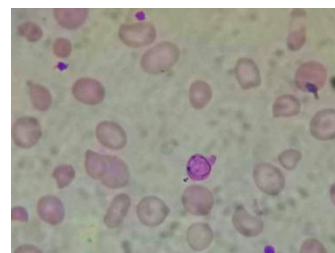


Fig. 3: Peripheral smear showing Macrocytic Anaemia with basophilic stippling (Leishman 100 x)

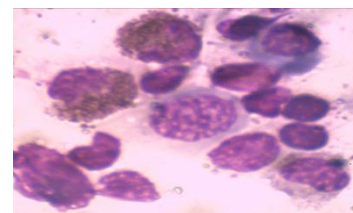


Fig. 4: Peripheral smear showing Bone marrow aspirate showing intermediate and late normoblasts with Megaloblastoid features (Leishman 100 x)

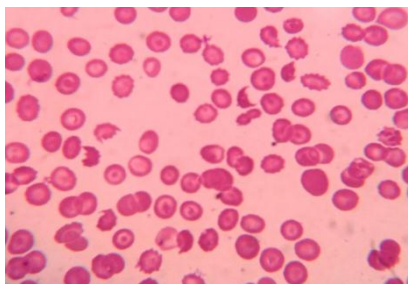


Fig. 5: Peripheral smear showing Normocytic Normochromic Anaemia with schistocytes indicating Hemolytic picture (Leishman 100 x)

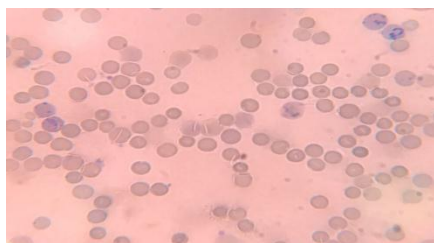


Fig. 6: Peripheral smear showing reticulocytes (Supravital stain 100 x)

Discussion

In India, about two billion people suffer from anaemia.¹ Being one of the developing countries, people of varied economic status are seen in India and so the diseases arising out of that also are varied. At one end of spectrum, we have started facing 21st century emerging problems like obesity and metabolic syndrome in urban population and at other end, we are still struggling with nutritional deficiency leading to anaemia in rural population which are malnourished people. Apart from nutritional deficiency, acute and chronic blood loss, destruction of red blood cells, dyserythropoiesis, inflammatory conditions and systemic disorders are also some of the uncommon causes of anaemia.

Thus, identifying the morphology and the aetiology of anaemia is necessary for the appropriate treatment of anaemia. This study provided the morphological pattern of anaemia in a rural tertiary care centre in South India by interpreting results from the automated hematology analyser and peripheral smear examination. Biochemical investigations of iron studies like serum Iron, ferritin, TIBC and serum vitamin B 12 and folic acid tests were carried out for further evaluation of anaemia. These findings were correlated with the clinical data like age, gender and severity of anaemia.

Total 300 cases of anaemia were studied in the department of Pathology at Mahatma Gandhi Medical College and Research Institute during a period between December 2015 and July 2017.

Comparison of Age-Wise Distribution of Anaemia Among Various Studies: In the present study, out of 300 patients, majority (96.3%) belonged to adult age group (> 15 years) and 3.6 % belonged to paediatric age

group. This could be due to susceptible age group to nutritional deficiency anaemia like teenage group due to growth spurt, pregnant women, geriatric age group due to underlying lifestyle or chronic diseases like diabetes which is more prevalent in Southern parts of India. Studies conducted by Ghalaut et al, Nazrin A. Qureshi et al and Gerardo Alvarez Urias et al also showed majority of percentage in adult age group susceptible to anaemia.^{3,5,6} Our study coincides with the above mentioned studies.

Sex Predilection: Females are more susceptible to anaemia because of the increased requirement of iron in them than in males.⁷

Study by Sandeep Barve et al from Gujarat (Western India) showed comparatively more frequency of anaemia among females compared to other studies.⁸ Percentage distribution of anaemia in male and female in our study showed similar findings with the study conducted by Banushree C et al as both the studies were conducted in Southern east coast of India.⁹

Comparison of Degree of Anaemia in Various Studies: In the present study, out of 300 patients, majority (68.3%) patients showed severe degree of anaemia. This could be due to ours being tertiary care centre where more sick patients are referred and mild and moderate degree get treated at Primary Health Centre or treated within community by anganwadi workers through various National programmes. Study by Ghalaut et al also showed majority (45 %) belonged to severe degree of anaemia,³ whereas other studies like Nasrin A. Qureshi reported mild degree of anaemia as the highest and the study of Gerardo Alvarez Urias et al reported moderate degree as the highest.^{5,6} Our study more or less correlates with the study conducted by Ghalaut et al. [Fig 5]

Comparison of Morphology of anaemia in Different Studies: In our study, microcytic hypochromic anaemia was the commonest morphological pattern (66%) of anaemia followed by dimorphic (23%), then macrocytic (8%) and least number of cases belonged to normochromic normocytic (3%). Most of the studies showed similar findings. [Fig. 6]

Comparison of Microcytic Hypochromic Anaemia with Various Studies: In the present study, microcytic hypochromic anaemia was the commonest morphological type of anaemia encountered. Similar findings were observed in the other studies, but the frequency of cases of microcytic hypochromic anaemia was higher in our study. It was found to be statistically significant (p value < 0.005). In our study, microcytic hypochromic anaemia showed reduced serum iron and ferritin levels, confirming the cause as iron deficiency anaemia. This could be due to dietary deficiency or chronic blood loss or both.

Majority of the patients, who had microcytic hypochromic anaemia, had low MCV and MCH like the other above mentioned studies. But MCHC in the

majority of the patients was within normal range in our study. Study conducted by Sandeep Barve et al also showed MCHC within normal range in the case of patients with microcytic hypochromic anaemia.⁸ This could be because the patients were on hematinic therapy and visited for the follow up. RDW was increased in the majority of these patients indicating severe anisocytosis in RBCs, pointing towards iron deficiency anaemia as a cause of hypochromic microcytic anaemia which was seen even in the study conducted by Banushree C.⁹ These findings were confirmed by further biochemical studies like Iron studies showing reduced serum iron and ferritin levels and increased TIBC levels in almost all patients of microcytic anaemia, thus ruling out other causes of hypochromic microcytic anaemia which won't respond to iron treatment. This was the benefit in our study which included Biochemical studies which were uncommon in other Indian studies. Also, two cases with presumptive diagnosis of Thalassemia trait based on iron studies were found which was not seen in other studies. Confirmatory thalassemia work-up was advised to these patients.

Comparison of Dimorphic Anaemia with Various Studies: The patients with dimorphic anaemia in our study showed reduced values of MCV, MCHC and MCH like the study conducted by Banushree C.⁹ RDW values were increased in these patients.

The percentage of cases of dimorphic anaemia (23%) in our study correlates with the study conducted by Banushree C (24%), as they share a common geographical distribution of Union territory of Puducherry whereas the other studies show low percentage of cases of dimorphic anaemia.⁹ [Fig. 6]. These cases underwent serum vitamin B 12, folic acid levels and Iron studies. Majority of the patients had reduced levels of serum Iron, ferritin and increased TIBC, few patients had reduced vitamin B 12 and folate levels and the rest showed normal levels of both vitamin B 12 and folic acid.

Comparison of Macrocytic Anaemia with Various Studies: The percentage of cases (8%) of Macrocytic anaemia also correlates with the other studies, except the study conducted by Sandeep Barve et al of Gujarat, which shows increased percentage (14%) of cases of Macrocytic anaemia. This could be because of vegetarian diet which is the staple diet of Gujarat which is deficient of B12 as compared to non-vegetarian diet of south India.⁸

Amongst the patients with Macrocytic anaemia, majority of patients had increased MCV, MCH was within normal range and MCHC was decreased, whereas the study conducted by Sandeep Barve et al showed increased MCV, MCH and MCHC.⁸ But the study conducted by Banushree C showed decreased MCH and MCHC with mildly elevated MCV amongst the patients with Macrocytic anaemia.⁹ Our study correlates with the study of Banushree C. RDW was

within normal range in all these patients. Bone marrow aspiration studies were conducted in few patients who showed Erythroid hyperplasia with Megaloblastic maturation. Further work up with serum vitamin B 12 and folate levels were done which showed low levels of both in majority of cases, three cases had reduced vitamin B 12 alone and two cases had normal levels of both the parameters. A study conducted in Maharashtra in which out of 100 patients, 10 % showed combined deficiency of vitamin B 12 and folate deficiency, although predominant deficiency was folic acid.⁸ In the present study combined deficiency of vitamin B 12 and folic acid was seen because of the dietary habits prevalent in this particular region of Pondicherry.

Comparison of Normocytic Normochromic anaemia with various studies: On comparison of percentage of cases of Normocytic Normochromic anaemia in our study (3%) with the other studies which ranged from 19 to 42%, percentage of cases were high in other studies. South India especially Pondicherry is not a prevalent geographical region for haemolytic anaemias like sickle cell anaemia or thalassemia as compared to central or western India which are endemic for sickle cell or thalassemia disease arising out of consanguineous marriage more among tribal population.¹⁰ Banushree C et al showed more cases of normochromic normocytic anaemia as compared to our study due to underlying chronic diseases.⁹

Among the patients with Normocytic Normochromic anaemia, MCV was within normal ranges, which was seen in other studies too. But the parameters like MCH and MCHC was below normal. On the other hand other studies had MCH and MCHC within normal limits.^{5,6,8} The study conducted by Banushree C et al showed reduced levels of MCH and MCHC like our study.⁹ It is because certain degree of microcytosis can be seen in anaemia of chronic diseases.¹¹ One of the common causes of Anaemia could be due to chronic disease like diabetes. It is said that Tamilnadu is the diabetic capital of India.¹² Diabetes is one of the common causes of chronic kidney disease resulting into normochromic normocytic anaemia.¹³

Limitations of the Study: The present study had some limitations. Although, Iron deficiency, Vitamin B 12 and folic acid deficiency were interpreted, further work up with recent parameters like transferrin percentage saturation, transferrin receptors and free erythrocyte protoporphyrin were not performed for complete evaluation of cause of these conditions. Being cross sectional study, in two presumptive thalassemia cases definitive thalassemia work up was beyond the scope of this study. Similarly, detailed evaluation of Normocytic normochromic and Haemolytic anaemia was not within the scope of this study. So exact cause remained undetected.

Conclusion

In our study, amongst all age groups, majority patients with anaemia were above 15 years with female predilection. In case of degree of anaemia, patient with severe degree of anaemia were observed the most. Among morphological types, microcytic hypochromic was the commonest type of anaemia in all age groups. In microcytic hypochromic, iron deficiency anaemia was found to be the cause after iron studies followed by two cases of presumptive thalassemia trait. Frequency of cases of microcytic hypochromic anaemia was higher in our study. dimorphic anaemia was the second most common morphological type of anaemia in which combined deficiency of Iron, B12 and folic acid were detected in some cases after further serum iron, B12 and folate studies.

Anaemia, although preventable, still remains one of the commonest causes of morbidity in 21st century India, especially among rural population. Dietary deficiency leading to various types of nutritional anaemia being the commonest cause, further morphological typing of anaemia supported by biochemical evaluation through Iron, B12 and folic acid studies are helpful for comprehensively evaluating other causes of anaemia as specific type of anaemia like thalassemia trait which needs specific treatment for better patient outcome.

References

1. WHO, UNICEF, and UNU, Iron Deficiency Anaemia: Assessment, Prevention and Control, A Guide for Programme Managers, WHO, UNICEF, UNU, Geneva, Switzerland, 2001. Available from http://www.who.int/nutrition/publications/micronutrients/anaemia_iron_deficiency/WHO_NHD_01.3/en/index.html.
2. M. B. Zimmermann and R. F. Hurrell. Nutritional iron deficiency. *The Lancet*. 2007;370:511-520.
3. P.S.Ghalaut, Mohini, Isha Pahuja, Naresh Gaur, Arvind Chahal, Suvrit Jain, et al. Study of morphological and etiological spectrum of in a tertiary care centre of north India. *International Journal of Current Research*. 2016;8(07):35300-03.
4. Singh T. Normal hematopoiesis and evaluation of bone marrow and peripheral blood film. In: Singh. T. *Atlas and Text of Hematology*. 3rd Ed, New Delhi: Avichal Publishing Company; 2014:1-50.
5. Nasrin A. Qureshi, Mohammed Abid Z. Chauhan, A.P. Goswami, S.K Suri. et al. Study of anemia and its correlation with Hematological parameters in patient of various age group. *IOSR Journal of Dental and Medical Sciences*. 2015;14(09):29-35.
6. Gerardo Alvarez-Uria, Praveen K. Naik, Manoranjan Midde, Pradeep S.Yalla, Raghavakalyan Pakam, et al. Prevalence and Severity of Anaemia Stratified by Age and Gender in Rural India. *Anemia*. 2014:5.
7. Helda Khusun et al. WHO hemoglobin cut-off points for the detection of anemia are varied for an Indonesian population. *J Nutr* 1999;129 (9):1669-1674.
8. Sandeep Barve, Darshana Patel, K. K. Shiromani, Ashish Jawarkar, et al. Role of RBC Count and RBC Indices in Diagnosing and Differentiating Anaemias Caused Due to

- Various Clinical Situations in a Tertiary Care Hospital in Vadodara, Gujarat. *Journal of Evidence based Medicine and Healthcare* .2015;2(45):8146-48.
9. Banushree C Srinivasamurthy. Spectrum of anaemia and red cell parameters of patients in a tertiary care hospital in rural South India. *Int J Biol Med Res*. 2013;4(1):2948-2951.
10. Dipty Jain, Vinit Warthe, Roshan Colah, Graham Roger Serjeant. Sickle Cell Disease in Central India: High Prevalence of Sickle/Beta Thalassemia and Severe Disease Phenotype. *Blood*. 2015;126:4588.
11. Firkin, F., C. Chesterman, D. Penington, and B. Rush. In: *Anemia in systemic disorders*. De Gruyter's Clinical Haematology in Medical Practice. 6th Ed, New Delhi: Oxford University Press; 2014:102.
12. Gupta SK, Singh Z, Purty AJ, et al. Diabetes Prevalence and its Risk Factors in Rural Area of Tamil Nadu. *Indian J Community Med*. 2010;35(3):396-399.
13. Nicholas J Radcliffe, Jas-mine Seah, Michele Clarke, Richard J MacIsaac, George Jerums, Elif I Ekinci. *J Diabetes Investig*. 2017;8(1):6-18.