STUDY OF HEMATOLOGICAL AND BIOCHEMICAL PROFILE IN CASES PRESENTING WITH SEVERE ANEMIA IN RURAL POPULATION

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ABSTRACT

Anemia is a worldwide health problem, but it is especially severe in developing countries like India. Anemia may have several causes, but iron deficiency anemia is the most frequent in underdeveloped countries like India. Using a variety of hematological and biochemical data, this research aims to identify the range and underlying causes of severe anemia in rural populations. In EDTA vacutainers and plain vials, 2 ml blood samples will be collected. Red cell indices (MCV, MCH, MCHC, PCV, RBC, RDW), platelet count (by automated analyzer), and a peripheral blood examination will all be *performed with a manual morphological analysis and a cell counter (Beckman coulter DXH700 automated hematology analyser) (by Leishman stained smears).*

Keywords: *Hemoglobin, Anemia, Microcytic, Hypochromic*

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I. INTRODUCTION

There is an increased risk of morbidity and death in all age groups due to anemia, making it a primary nutritional concern in developing countries like India. Hematological markers, such as hemoglobin, red blood cell indices, Peripheral Blood Smear test, and biochemical parameters, are helpful in the diagnosis of anemia since the clinical presentations of various forms of anemia are comparable. Anemia is defined medically as a decrease in RBC count, hemoglobin concentration, or hemoglobin's capacity to bind oxygen. Estimates that utilized Hb as a proxy to estimate the prevalence of IDA are primarily responsible for the widespread misconception that iron deficiency (ID) is the leading cause of anemia globally. However, there are several causes of anemia, including deficits in folic acid, vitamin B12, and vitamin A; parasite infections, including malaria and helminths; and chronic inflammatory disorders.

As the condition progresses, anemia has devastating long-term effects. It will adversely affect children's and teenagers' physical development and ability to pay attention, remember information, and do well in class [1]. The development of menarche is also delayed, and the immune system is negatively impacted, leading to recurrent infections [2]. Preterm delivery, low birth weight, and infant mortality are all linked to anemia in pregnant women [3]. Adults who suffer from anemia have a decreased ability to do their jobs, which has social and economic repercussions for them and their loved ones. It is possible to categorize anemia based on its pathophysiology, red cell shape, and clinical presentation. A speedier diagnostic method is possible in clinical use by categorizing based on fundamental red cell morphology criteria, such as mean corpuscular volume (MCV) [4]. When measuring red blood cells, scientists use two different methods: mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH). MCV measures a red blood cell's average size and volume in femtoliters (fl), while MCH measures the average hemoglobin per erythrocyte in picograms (pg). The hemoglobin concentration in a fixed volume of packed red blood cells is reported as mean corpuscular hemoglobin concentration (MCHC) g/dl [5]. Microcytic hypochromic anemia (MCH), normocytic anemia (NCH), and macrocytic anemia (MCV) are the morphological subtypes of anemia [6]. Chronic iron-deficiency anemia, anemia of chronic illness, sideroblastic anemia, and thalassemias are the most common causes of microcytic anemia, although it may also occur in other diseases. Macrocytic anemia occurs when the average volume of red blood cells is higher than usual (MCV >100 fl), as may happen in conditions like persistent drinking, hepatic insufficiency, and folate or vitamin B12 deficiency. Nutritional inadequacy, renal failure, and hemolytic anemia are all potential causes of normocytic anemia, characterized by a low hemoglobin but an MCV within the normal range of 80 to 100 fl.

There is a wide variety of community-level causes of anemia. Malaria and parasite diseases, nutritional deficits including anemia, hemoglobinopathies, enzymopathies, and membranopathies of red blood cells are all examples. Chronic disease/inflammation-related anemia and bone marrow loss may also play a role, but to varying degrees. An iron deficiency is the most common cause of anemia in the world. However, the other types of anemia may occur side by side with iron deficiency anemia [7]. Variations in anemia prevalence exist between racial and ethnic groupings. The areas of Central, West, and East Sub-Saharan Africa, as well as South Asia, have the highest recorded rates of anemia [8]. According to global studies of anemia prevalence, pregnant women and young children seem to be the most at risk. The World Health Organization (WHO) reports that anemia affects 40.0% of pregnant women globally and 42.0% of children less than 5 years old. Researchers in India observed high rates of anemia among children 6–59 months old (58.4%), women 15–

49 (53.1%) who were not pregnant and women 15–49 (50.3%) who were pregnant in 2015–2016 [4]. Also, 52.6% of children in Nepal between the ages of 6 and 59 months were anemic in a nationwide study [9]. As the most common anemia in the older population, anemia due to chronic disorders ranks second globally [10]. Even though CKD is the leading cause of anemia of chronic illness, other conditions such as IBD, autoimmune disorders, and long-term infections may also contribute to the condition. The prevalence of anemia in CKD patients is approximately 75.0% [11] by considerable research. Evidence suggests that the elderly have a greater frequency of anemia [12]. A few research organizations have only studied the frequency of anemia among the elderly in Asia and the variables that contribute to anemia. Nearly half (47.7%) of the elderly population in Northeastern Thailand had anemia [13]. According to a research done on residents of Delhi's retirement communities, anemia affects 68.7% of the population; the rate is somewhat higher for women (70.9%), at 65.1%, than for males. Research shows that around 50% of individuals with malignancies are anemic [14]. In 2020, the estimated number of new cancer cases worldwide was 50,550,287, according to the World Health Organization [15]. Therefore, it is clear that there may be a high rate of cancer-related anemia in the general population.

Hematological profile: Hematological profile is the blood tests panel that gives cells type in patient’s blood specimen. These type of cells are white blood cells (WBC), red blood cells (RBC) and platelets (Thrombocytes). A normal adult have about 5 liter of blood (2 liter of cells and one liter of plasma approximate). Each and every one kind of blood cells are produced in bone marrow’s hematopoietic stem cells.

II. AIM & OBJECTIVES OF THE STUDY

Aim: Examining a variety of hematological and biochemical markers, this research seeks to identify the range and underlying causes of severe anemia in rural populations.

Objectives: To characterize the hematological and biochemical features of severe anemia in rural areas.

III. LITERATURE REVIEW

Red blood cells (RBCs) contain a metalloproteinases called hemoglobin (Hb) that shuttles oxygen to tissues and carbon dioxide back to the lungs [18, 19]. A worldwide epidemic, anemia is characterized by a lack of hemoglobin in the blood. About 25% of people worldwide are affected by this illness [20], with most cases occurring in developing nations and disproportionately affecting women and children. Reduced academic and occupational performance are only two of anemia's many detrimental effects on society and the economy [20]. Symptoms of anemia range from modest weakness, weariness, and dizziness to the potentially fatal cardiovascular system collapse in severe instances [21, 22].

The Global Burden of Disease (GBD) Study [23] done in 2013 by the Institute for Health Metrics and Evaluation found that anemia affects 27.0% of the world's population (1.93 billion people) worldwide. From 1993-2005, researchers found that anemia afflicted 41.8% of expecting mothers, 30.2% of non-pregnant women, 47.4% of preschoolers, and 25.4% of elementary schoolers (about 56,400,000, 468,400,000, 293,100,000, and 304,600,000 persons, respectively) [24].

Benjamin Onyema Aledo et al [25] reported on 43 participants. Study results demonstrated on control and test participant. The hemoglobin concentration of control and test population was 10.46 gm% and 13.64 gm% respectively, whereas hematocrit was 33.86% and 41.05% respectively for control and test population. 34.00 mm/hour and 7.85 mm/hour mean ESR (erythrocyte sedimentation rate) were found in females in the test and control groups. In these observation, a significant ($P<0.05$) reduction in thrombocytes, hematocrit, HB, and increment of total leucocyte count and ESR.

Oluwasola O. Obebeet et al [26] resulted among 130 pregnant subjects that the occurrence of malaria was 14.6%. Prevalence of malaria parasitemia was highest (68.4%) in the 2nd trimester. The mean of hemoglobin (9.46 gm%) in asymptomatic malaria gestational women was significantly lower than that of their non-malaria infected (10.35 gm%) pregnant women. There was a significant variation in the PCV (31.42%) of asymptomatic malaria pregnant women compared to their nonmalaria (33.95%). Significant variation in total WBCs count linking two clusters of persons The mean Platelets, R-B-Cs, MCV, MCH, were also found to be lower in asymptomatic Plasmodium falciparum-infected pregnant females (200.26 × 103/µl, 3.65, 86.84 fl, and 26.21 pg) compared to their negative individuals (221.58 × 103/µl, 3.87, 88.86 fl and 27.14 pg) correspondingly but this difference was not significant.

Huma Hameed et al [27] depicted anemia occurrence in gestational subjects as 65.4%. This regional study from Pakistan showed that the 6.4% pregnant women had high grade anemia, and middle grade anemia found in 19.2% in females' gestational. In contrast, low grade anemia observed in the 39.8% study population of the gestational women. Pregnant women who had anemia and daily intake of three or more cups of tea have a significant association with pregnancy and anemia. Related important co-relation was also found between anemia, vegetarian food and low grade diet. "The maximum anemic females were in the age group of 35>40. Over-all 17.7% foetal mortality was recorded with significantly highest among anemic group."

Ezra Belay et al [28] resulted among 196 pregnant women that after the smallest amount of one-month IFA supplementation sufficient hemoglobin response by 11 gm% and 51.5% gestational women had hemoglobin more than 11 gm% and feedback noted in remaining 48.5% participant of the research. After Iron and folic acid IFA supplementation the anemic rate of the study population dropped and stay at 13.8% from 25.5%. hematocrit was also made positive response in both gestational and non-gestational women.

Atiyeh Namazi [29] observed that anemia during gestational time is a public health anxiety worldwide. Iron deficiency is a primary contributor to anemia that affects more than half a billion females of reproductive age and has a occurrence of 17.4% in industrialized countries, whereas 60% in under-developed countries. Significant increase in plasma quantity than erythrocyte number during pregnancy results in relative anemia. Iron deficiency is connected with adverse health consequences for both women and their kids like low birth weight, pre-term birth, maternal cardiac failure, hemorrhage, infection, miscarriages, and stillbirths. There is also statistically meaningful co-relation between anemia and residence, gravidity, pregnancy interval, and malaria illness during pregnancy. Oral iron supplements, pills, capsules, drops, and extended-release tablets are often prescribed to treat anemia. WHO and CDC recommend 60 mg/daily and 30 mg/daily for all pregnant women.

In thestudy, Hb linked favorably with RBC, HCT, MCV, MCH, and monocyte. Evidence suggests that total Hb, RBC, and HCT are highest during birth [30]. By the time a person reaches puberty, their Hb and RBC levels have increased to adult levels [31]. Homozygous sickle cell anemia patients had aberrant hemoglobin synthesis, and Maude et al. [32] discovered that RBC correlated favorably with total Hb in these individuals. HCT has been shown to correlate well with Hb in most cases, but to be even less sensitive for iron insufficiency than Hb [33]. Since Hb concentration fluctuates in tandem with cell volume, the observed positive correlation between Hb and MCV predicts a lower prevalence of macrocytic anemia among the research group. Red blood cells (RBCs) divide twice to produce four new RBCs in the bone marrow, and each set of offspring is smaller than the original. Reduced cell division and Hb biosynthesis result in larger-than-normal or macrocytic erythrocytes [34]. An elevated mean corpuscular volume (MCV) contributes to this phenomenon. In a study of senior Pakistani men, Khan et al. [35] discovered a strong correlation between Hb and MCH. MCH decreases due to decreased Hb production in iron deficit [36]. Ferroportin is a transmembrane protein that moves iron from enterocytes and monocytes/macrophages into the bloodstream [37]. Compared to healthy controls, ferroportin mRNA expression was considerably lower in monocytes of anemic people [38]. Importantly, higher iron storage in monocytes of anemia of chronic illness patients was matched by reduced expression of ferroportin, as measured by hyperferritinemia. A decrease in ferroportin expression and the associated reduction in cellular iron export would raise intracellular iron levels, which will interfere with the process of erythropoiesis, resulting in a drop in monocyte expression and a corresponding fall in hemoglobin concentration (Hb).

As expected, RBC was linked to HCT and monocyte, but inversely connected to MCH. Anemia is decreased RBC count and hemoglobin concentration (HCT) [39]. There was a robust relationship between venous HCT levels and total RBC volume in the bloodstream [40]. The activation state of human monocytes suggests a possible role of oxidative stress in these children [41]. Under autologous experimental circumstances, oxidative stressed erythrocytes in blood significantly exacerbates cytokine production. Reduced red blood cell count (RBC), hemoglobin (Hb), and hematocrit (HCT) and elevated mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) are hallmarks of anemia [42].

In this retrospective analysis, researchers looked at 109 kids diagnosed with pancytopenia. Bicytopenia and pancytopenia in children younger than 14 for at least 2 years were analyzed. Hemoglobin levels below 10%, absolute neutrophil count below 1500, and platelet count below 100,000 all indicate pancytopenia. The whole hematological and clinical parameters of the patient were documented. Megaloblastic anemia was the leading cause of pancytopenia, affecting children of 31 (28.4%). Aplastic anemia (20%) and acute leukemia (21%), which may manifest as acute lymphoblastic leukemia, acute myeloblastic leukemia, or myelodysplastic syndrome, are other prevalent causes. Twenty-three (21% of the sample) children had pancytopenia due to illnesses such as malaria, kala-azar, and bacterial septicaemia. There were other cases of CMV, TB, and infectious mononucleosis. Severe pancytopenia affected 21 children (20%). About two-thirds of all patients with severe pancytopenia had either acute malignancy or aplastic anemia as the underlying cause. For example, megaloblastic anemia, kala-azar, sepsis, and acute leukemia cause severe anemia (mean Hb 7g%). A low median hemoglobin characterized megaloblastic anemia. Petechiae, bruising, and ecchymosis (excessive bleeding under the skin) were the most prevalent forms of external bleeding. Hepatomegaly and splenomegaly were seen in 14 of the 28 individuals (45%) with megaloblastic anemia. Infantile tremor syndrome was present in 3% of megaloblastic anemia patients. It is now well accepted that megaloblastic anemia caused by a lack of vitamin B12 or folic acid significantly contributes to cytopenias. Megaloblastic anemia was the leading cause of pancytopenia, even though leukemia and aplastic anemia also contribute to the condition.

Poor dietary iron intake, persistent blood loss, blood loss owing to intestinal worm colonization (i.e., hookworm infection), and iron malabsorption are all common causes of iron deficiency anemia [43], especially in low and medium income countries. Because of blood loss at tumor sites, malignant invasion of normal tissue and bone marrow, and inflammation-induced sequestration/decreased use of iron, cancer may produce functional iron shortage [44]. In malaria-endemic regions, anemia is also common. [45] Malaria causes anemia in young children in areas where transmission is intense. Malaria has been linked to poor birth outcomes [46] and maternal anemia at all transmission stages throughout pregnancy. Hemolysis of red blood cells (RBCs) and severe, chronic anemia may be caused by inherited hemoglobin diseases including SCD and thalassemia [47].

Early diagnosis and prompt treatment are crucial to reducing the effects of anemia [48]. Timely and correct diagnosis of the particular cause of anemia is necessary for effective therapy [49]. If the reason of anemia is misdiagnosed, the unfair treatment will be administered, which might have disastrous consequences [50]. Micronutrient supplementation is effective in treating conditions like iron deficient anemia. Iron supplements are recommended by the World Health Organization [51], but only in areas with malaria control measures. Patients with sickle cell disease (SCD) and thalamic patients with an iron homeostasis imbalance are also at risk for iron overload due to hemolysis and repeated transfusions [52]. Because of this, prompt and correct diagnosis of anemia and determination of its cause are paramount.

IV. MATERIAL AND METHODS

Study Design: A study was conducted from October 2021 to May 2022 in the Department of Pathology at SMCW & SUHRC, Lavale, Pune. This was questionnairebased research to test patients' understanding about anemia.

Inclusion criteria: All the patients presenting with severe anemia. (Below 7.0 gm%)

Exclusion Criteria: Patients with a history of coagulation and bleeding problems, patients with malaria-related anemia, patients taking iron or multivitamins, patients with any chronic condition, and patients who are pregnant or breastfeeding.

Sample Size: Samples received and analyzed by the Central Clinical Laboratory (CCL) during eight months to be included in the study.

Methodology: EDTA Vacutainer and two milliliters of blood drawn in plain vials. Red cell indices (MCV, MCH, MCHC, PCV, RBC, RDW), platelet count (by automated analyzer), and a peripheral blood examination will all be performed with a manual morphological analysis and a cell counter (Automated Haematology Analyzer) (by Leishman stained smears). Check iron and ferritin levels, as well as your vitamin B12 and folate levels, to see how your body is doing a biochemical analysis of bone marrow (where necessary).

V. STATISTICAL ANALYSIS

- Mean and standard deviation will be used to summarize the data.
- To determine statistical significance, we shall use the paired t test.
- SPSS will be used in its most recent form for all data analysis.
- A p-value less than 0.05 will be regarded as statistically significant.

VI. RESULTS AND DISCUSSION

Anemia is a global health problem with significant impact on health and wellbeing. Worldwide around 1.62 billion people are affected with anemia, which is around 24.8% of the world population. Despite recent economic growth and awareness among people, the prevalence of anemia among various age groups persist in India and is an vital contributor to extreme health conditions such as fatigue, malaise, neurological dysfunction, poor focus and attention. In earlier research, poor iron and other nutrient consumption, decreased vitamin C intake, recurrent child carrying, breastfeeding, and insufficient availability to nutritional supplements after menarche and throughout pregnancy have been described as potential causes of anemia in the Indian population.

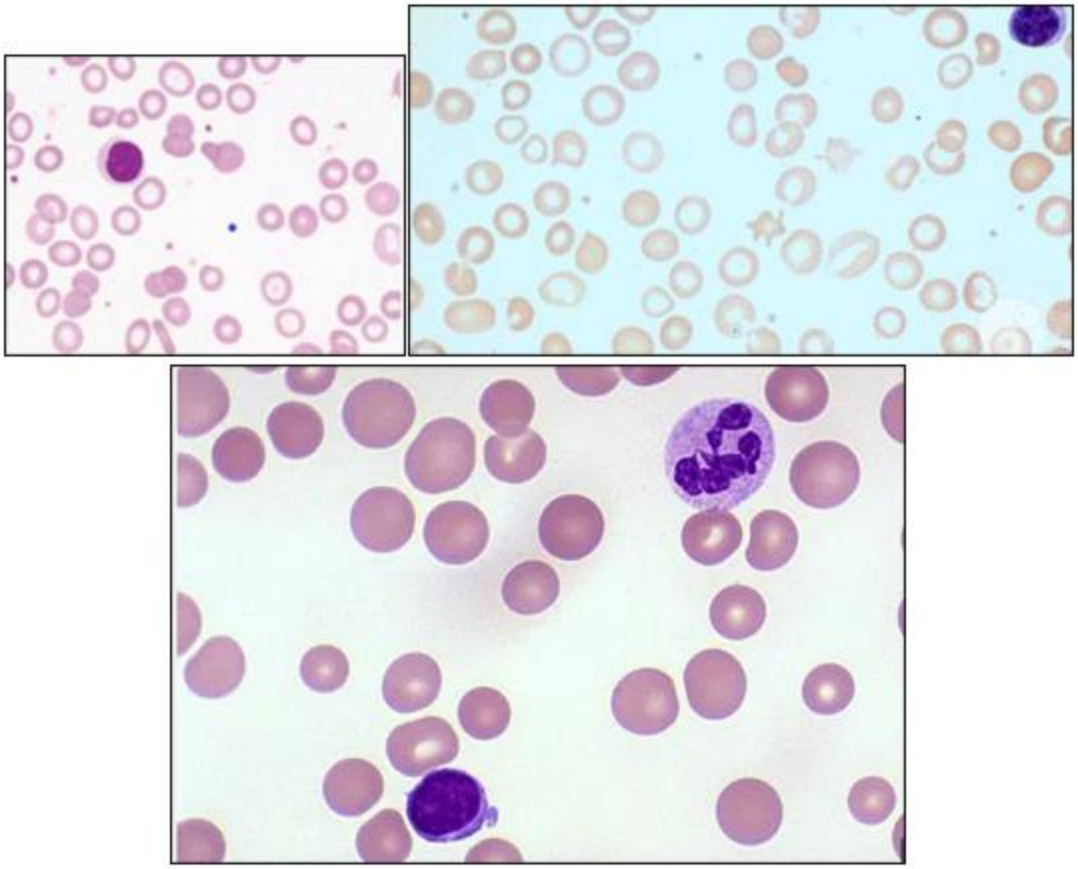


Fig 1 - Peripheral blood smear findings in Anemia

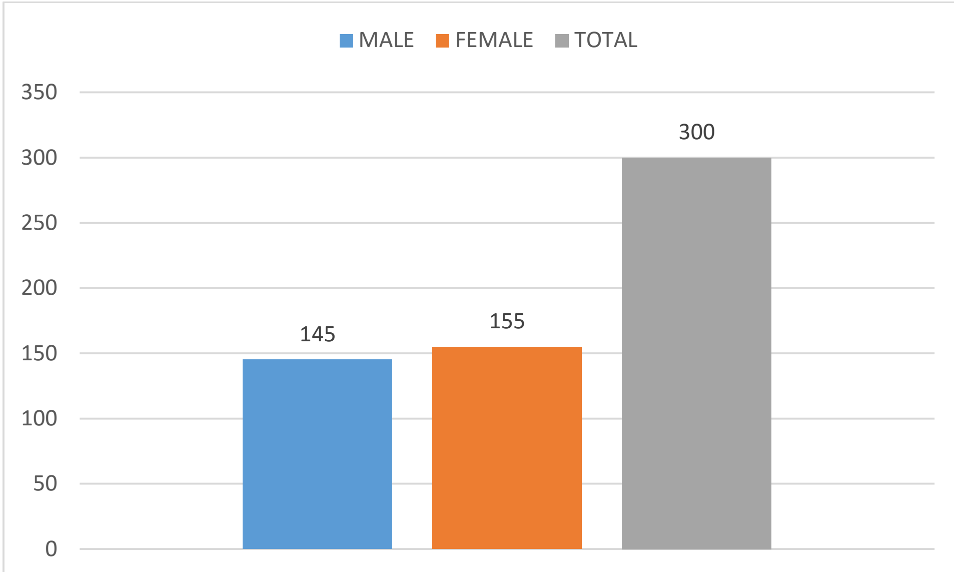
Most of the previous research had employed the WHO-recommended age-, gender-, physiological-state-, and altitude-adjusted cutoff thresholds for anemia. Though most papers we reviewed met these criteria, we found a few outliers. This heterogeneity may confound the prevalence of anemia findings presented in this research.

Analyzing the hematological and biochemical profile of severe anemia patients in rural populations was conducted from October 2021 to May 2022 in Pune. It is a cross-sectional, hospital-based study wherein 300 patients with severe anemia with below 7.0 gm% data have been analyzed. The shortlisting of the patients is done via patient profile. The study excluded patients with anemia due to malaria or chronic illnesses or a history of coagulation and bleeding disorders.

A 2-milliliter blood sample was collected from the patient in EDTA vacutainers and plain vials. Blood was examined manually and with a cell counter (Beckman coulter DXH700 automated hematology analyzer) to determine indices for red blood cells (MCV, MCH, MCHC, PCV, RBC, RDW), as well as to determine the number of platelets in the blood (also by automated analyzer) (by Leishman stained smears). Moreover, the chi-square test, with a 5% significance threshold, has been used to examine the gender differences in patients' reports of anemia.

Furthermore, anemia is a fatal condition for 40.3% of patients. On the other hand, 17% of patients are not sure about the consequences caused by anemia. The remaining 42.6% of patients said anemia is not a deadly condition. In addition, the patients said the cause of anemia is because of poor diet (35%), intestinal worm infestation (29.3%), and severe blood loss (13%), which differs at gender level (χ^2=18.740, sig=0.000, ≤ 0.05) and their prevention is via intake of iron-rich foods

(26.6%), consumption of caffeine products (24.3%), and the intake of vitamin-c and folic acid-rich foods (27.3%).



Gender distribution of the study population (N=300)

Table 1 Patients Information about Anemia at the Gender level

Patients Information about Anemia		Gender: Male	Gender: Female	Total	Chi-Square Value	df	Sig.
heard about anemia	Yes	97 (32.3%)	109 (36.3%)	206 (68.6%)	.409ª	1	0.523
	No	48 (16%)	46 (15.3%)	94 (31.3%)			
Total		145 (48.3%)	155 (51.6%)	300 (100%)			
anemia is a fatal condition	Yes	54 (18%)	67 (22.3%)	121 (40.3%)	1.241ª	2	0.538
	No	64 (21.3%)	64 (21.3%)	128 (42.6%)			
	May be	27 (9%)	24 (8%)	51 (17%)			
Total		145 (48.3%)	155 (51.6%)	300 (100%)			
usual range of hemoglobin in females	12.0-15.0 g/ dl	48 (16%)	41 (13.6%)	89 (29.6%)	9.143ª	3	0.027
	15.0-18.5 g/dl	38 (12.6%)	25 (8.3%)	63 (21%)			
	8.0-11.0 g/dl	42 (14%)	66 (22%)	108 (36%)			
	6.0-8.0 g/dl	17 (5.6%)	23 (7.6%)	40 (13.3%)			
Total		145 (48.3%)	155 (51.6%)	300 (100%)			
causes anemia	Poor diet	46 (15.3%)	59 (19.6%)	105 (35%)	18.740ª	3	0.000
	Intestinal worm infestation	59 (19.6%)	29 (9.6%)	88 (29.3%)			
	severe blood loss	13 (4.3%)	26 (8.6%)	39 (13%)			
	All the above	27 (9%)	41 (13.6%)	68 (22.6%)			

Total		145 (48.3%)	155 (51.6%)	300 (100%)			
prevent anemia	Intake of iron-rich foods	36 (12%)	44 (14.6%)	80 (26.6%)	.620[a]	3	0.892
	Consumption of caffeine products	37 (12.3%)	36 (12%)	73 (24.3%)			
	Intake of Vitamin C & folic acid-rich foods	41 (13.6%)	41 (13.6%)	82 (27.3%)			
	Both first and third	31 (10.3%)	34 (11.3%)	65 (21.6%)			
Total		145 (48.3%)	155 (51.6%)	300 (100%)			
common symptoms of anemia	Pale skin	52 (17.3%)	44 (14.6%)	96 (32%)	9.558[a]	3	0.023
	Tiredness	27 (9%)	45 (15%)	72 (24%)			
	Both A and B	48 (16%)	36 (12%)	84 (28%)			
	Icterus	18 (6%)	30 (10%)	48 (16%)			
Total		145 (48.3%)	155 (51.6%)	300 (100%)			
heavy menstrual bleeding cause anemia	Yes	58 (19.3%)	66 (22%)	124 (41.3%)	.398[a]	2	0.820
	No	47 (15.6%)	51 (17%)	98 (32.6%)			
	May be	40 (13.3%)	38 (12.6%)	78 (26%)			
Total		145 (48.3%)	155 (51.6%)	300 (100%)			
foods rich in iron content	Banana	28 (9.3%)	33 (11%)	61 (20.3%)	2.105[a]	3	0.551
	Pomegranate	28 (9.3%)	21 (7%)	49 (16.3%)			
	Honey	43 (14.3%)	45 (15%)	88 (29.3%)			
	All the above	46 (15.3%)	56 (18.6%)	102 (34%)			
Total		145 (48.3%)	155 (51.6%)	300 (100%)			
foods that worseness anemia	Tea and coffee	26 (8.6%)	30 (10%)	56 (18.6%)	1.254[a]	3	0.740
	products made up of Honey	42 (14%)	44 (14.6%)	86 (28.6%)			
	Red meat	50 (16.6%)	59 (19.6%)	109 (36.3%)			
	Nuts and seeds	27 (9%)	22 (7.3%)	49 (16.3%)			
Total		145 (48.3%)	155 (51.6%)	300 (100%)			
hemoglobin level is 10mg/dl, are you considered as anemia or not	Yes	43 (14.3%)	57 (19%)	100 (33.3%)	2.359[a]	2	0.307
	No	65 (21.6%)	57 (19%)	122 (40.6%)			
	May be	37 (12.3%)	41 (13.6%)	78 (26%)			
Total		145 (48.3%)	155 (51.6%)	300 (100%)			

The usual range of hemoglobin in females is 12.0-15.0 g/ dl (29.6%), 15.0-18.5 g/dl (21%), 8.0-11.0 g/dl (36%), and 6.0-8.0 g/dl (13.3%), which is significant at gender level ($\chi2=9.143$, sig=0.027, ≤ 0.05). Besides that, the common symptoms of anemia is pale skin (32%), tiredness (24%), and icterus (16%), which is also significant ($\chi2=9.558$, sig=0.023, ≤ 0.05) at the gender level. As per 41.3% of patients, heavy menstrual bleeding cause anemia, which is not sure by 26% of patients. That is why at the gender level, heavy menstrual bleeding cause anemia is not significant. That is why patients with anemia should avoid Tea & coffee (18.6%), products made up of honey (28.6%), red meat (36.3%), and nuts and seeds (16.3%). Ideally, patients should take food like a banana (20.3%), pomegranate (16.3%), and raw honey (29.3%). These differences are not significant at the gender level but essential to care for. Further, according to 33% of the patients, if the hemoglobin level is 10mg/dl, it is considered to be anemia.

In the table-2, at gender level patients hematological and biochemical profile have been assessed. The values of hematological parameters are as follows Hb (1.7-10.4) (g%) (t-value=-33.4, sig=0.00), TLC (880-19000) (cu.mm) (t-value=31.04, sig=0.00), Platelet Count *10^3 (10-890) (t-value=29.09, sig=0.00), RBC * 10^6 (0.64-4.8) (t-value=-104., sig=0.00), MCV (fl) (58.6-127) (t-value=66.59, sig=0.00), MCH (pg) (12.8-40) (t-value=27.08, sig=0.00), MCHC (%) 22.6-35 (t-value=62.84, sig=0.00), and RDW (%) 13.2-26.9 (t-value=29.47, sig=0.00) and the biochemical values are Vitamin B12 (180-914) pg/ml (t-value=41.05, sig=0.00), Folate (3-20) ng/ml (t-value=3.464, sig=0.00), Ferritin (11-336.2)ng/ml (t-value=29.29, sig=0.00), and TIBC (250-400) pg/dl (t-value=85.26, sig=0.00). Both the parameter values are less than 0.05; hence it can be inferred that the results are significant. In SPSS, one sample t-test is used to assess the results at a 5% significance level.\

Table-2 Mean ± Std. Deviation of the hematological and biochemical profile at the Gender level

Parameters		Male	Female	t-Value	Df	Sig
Hematological Parameters	Hb (1.7-10.4) (g%)	6.641 ± 2.854	6.4 ± 2.825	-33.47	299	0.00
	TLC (880-19000) (cu.mm)	10734.24 ± 5698.4	9674. ± 5626.036	31.04	299	0.00
	Platelet Count *10^3 (10-890)	433.8 ± 242.3	452.2 ± 269.9	29.10	299	0.00
	RBC * 10^6 (0.64-4.8)	3.027 ± 1.495	2.903 ± 1.497	-104.67	299	0.00
	MCV (fl) (58.6-127)	86.67 ± 19.96	86.41 ± 18.89	66.59	299	0.00
	MCH (pg) (12.8-40)	28.41 ± 10.75	28.25 ± 10.17	27.09	299	0.00
	MCHC (%) 22.6-35	29.12 ± 4.765	29.30 ± 4.740	62.84	299	0.00
	RDW (%) 13.2-26.9	20.95 ± 5.444	21.16 ± 5.232	29.48	299	0.00
Biochemical Parameters	Vitamin B12 (180-914) pg/ml	555.5 ± 223.1	555.0 ± 235.4	41.05	299	0.00
	Folate (3-20) ng/ml	13.54 ± 7.052	13.20 ± 6.671	3.46	299	0.00
	Ferritin (11-336.2)ng/ml	175.8 ± 97.28	172.8 ± 94.94	29.30	299	0.00
	TIBC (250-400) pg/dl	328.2 ± 65.85	337.5 ± 64.49	85.27	299	0.00

Table 3: biochemical parameters with correlation study

BIOCHEMICAL PARAMETERS	NORMAL REFERENCES LEVEL	NO. OF CASES WITH DECREASE VALUE	NO. OF CASES WITH INCREASE VALUE	NO. OF CASES WITH NORMAL VALUE	TOTAL NO. OF CASES	% OF CASES CORRELATION WITH PBS	% OF CASES CORRELATION WITH PBS

Vitamin B12	(180-914) pg/ml	136	103	61		20.34	79.66
Folate	(3-20) ng/ml	171	56	73		37.56	62.44
Ferritin	(11-336.2)ng/ml	89	116	95	300	62.9	37.1
TIBC	TIBC (250-400) pg/dl	62	71	167		14.1	85.9

VII. CONCLUSION

The early identification and prevention of severe anemia may be achieved using hematological and biochemical testing. Measures of providing enough nutrition and management may supplement preventative programs to reduce the prevalence of severe anemia. The results of this study suggest that primary health care providers should prioritize the prevention and early diagnosis of anemia because this condition has been linked to a lag in preschoolers' psychomotor development. Most instances of anemia may be detected as early as possible with the use of appropriate screening and subsequent preliminary diagnostic modalities. Accurate diagnosis of exact etiology of anemia is paramount for effective treatment and best results. Malnutrition-related anemia is quite common in our nation.

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