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STUDY OF HAEMOGLOBIN LEVELS IN THE ANEMIC PATIENTS OF DISTRICT SHAHEED BENAZIR ABAD, SINDH

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Abstract

Anemia, a widespread and significant health concern, is a condition resulting from a lack of iron and is considered a serious global problem within the public sector. Anemia is diagnosed when hemoglobin levels fall below 11 g/dL in children aged 6–50 months, and below 11.5 g/dL in children older than 5 years. The abnormal erythrocytes cells affects to the blood smear slides due to depend on the hemoglobin level or ranges for diagnostics and evaluating stages of anemia include Hemoglobin (HB; %), Haematocrit (HCT; %), Red blood cells (RBCs; %), Mean corpuscular volume (MCV; %) and mean corpuscular hemoglobin concentration (MCHC; %) used to measure the hemoglobin level in the patients, gender-vice with different age groups. Blood samples (n=250) of male and female were collected from the Pathology and Diagnostic Research Laboratory, at jhooly lal diagnostics laboratory and Al- Hyder diagnostic laboratory Nawabshah, Shaheed Benazir Abad from 1 August to 30 October in 2023 year. Samples were collected from the anemic patients by normal ranges compare to abnormal range according to the age groups, respectively. In these parameters shows different range of HB% level in anemic patients by RBCs range (4.7 to 6.1 Mil/cumm), HCT range (41 to 50 %), Hb level rang (14 to 18 g/dl), MCV range (80 to 100 fl), MCH range (27 to 31 pg) and MCHC range (32 to 36 G/dl). Data are divide into four age groups, 1-20, 21-49, 41-60 and 61-80 with gender differences ranges in these age groups shows the different ranges in the anemia affected patients including abnormal anemia affected erythrocytes cells are describe the three categories microcytic, macrocytic and normocytic and normochromic also shows as the size shapes and color in the inside red blood cells from patients samples. Our study revealed a significant variation of Anemia in patients with low Hb levels poses a multifaceted challenge, necessitating a comprehensive approach for accurate diagnosis and effective management.

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INTRODUCTION

Chapter 1 Introduction

This chapter motivates and supports the nation that anemic patients and its associated health issues are a major public health concern around the world. Anemia is the most hematological disorder of due to iron deficiency in anemic patients. To diagnose iron deficiency anemia, compare the lower hemoglobin than normal hemoglobin levels in anemic patients. Anemia is formally diagnosed when the hemoglobin concentration in the blood falls below the age- and sex-specific threshold. The goal of this current study is to determine how common hemoglobin levels associated with differences in quantitative measurements of anemic patient are individuals.

Anemia, a widespread and significant health concern, is a condition resulting from a lack of iron and is considered a serious global problem within the public sector (WHO 2012; Camaschella 2015). Globally, approximately 2 billion individuals experience iron deficiency anemia (IDA), making it the most prevalent nutritional problem worldwide. The majority of these individuals, accounting for 89%, reside in developing countries (Kassebaum 2016). According to the WHO (world health organization), Most of the African and South Asian nations as well as some of the nations in East Asia and the Pacific have a high anemia prevalence. Although Africa has the high prevalence of anemia, the Asia has anemic children of proportion is high (Ewusie et al. 2014). Giving to Global Burden of Disease (GBD) models there were 8.3 million (95% CI: 5.5 to 12.1 million) years lived with disability worldwide among children under 5 years old due to anemia in 2019, the majority of which (7.8 M, 95% CI: 5.2 to 11.5 million) were due to moderate or severe anemia (Zheng et al. 2021). Anemia can have many causes, such as nutritional deficiencies, infections and hemoglobinopathies (group of blood disorder) (Engle et al. 2017). Iron deficiency is one of the largest causes of anemia (Kassebaum 2016), but studies estimating the proportion of anemia due to iron deficiency have highly variable results partly because diagnosing iron deficiency is difficult. A meta-analysis of 23 national surveys found that 25% of anemia among children is associated with iron deficiency with high heterogeneity (range: 0.3%–51%) (Petry et al. 2016).

It is characterized by anemia iron deficiency leads to decrease hemoglobin synthesis for microcytic and hypochromic RBC production. Reduced iron intake or absorption and an increase in iron requirement during periods of significant blood loss are two effects of iron deficiency anemia (IDA) (Kumari et al. 2017). Low levels of hemoglobin have negative effects on cognitive and motor development, leading to fatigue and decreased productivity (Balarajan et al. 2011). While elevated hemoglobin levels can have certain negative effects (Murphy et al. 1986). Many of arise low range of concentrations and associated with poorer outcomes decrease. Limited another concentration could be effect corresponding from mild anemia to ranging (Kozuki et al. 2012). Hemoglobin level is still within the normal range until the development of the iron deficiency anemia (IDA) stage. To the point that depletes iron storage levels they can no longer support the hemoglobin production and generate enough red blood cells (RBCs). Iron deficiency damage red blood cell synthesis leading to anemia cause decrease production of hemoglobin (Silberstein et al. 2017). Furthermore, a full blood count assists in establishing the precise level of anemia (Munro et al. 2014). The majority, over two-thirds, of the body's iron is found in erythroid precursors and mature red blood cells (RBCs) as hemoglobin (Hb) (Andrews 1999). As a result of severe iron deficiency, there can be a decrease in hemoglobin levels, which hinders the production of red blood cells (RBCs). This, in turn, leads to the development of normocytic or microcytic hypochromic anemia (Mansour et al. 2021). It is a hypochromic and microcytic anemia with decreased MCV and MCH as well as Hb values for sex and age below the normal range. For development of the pregnancy, newborn, and child, iron is a crucial nutrient (Cerami 2017). Hemoglobin cycling patterns, in which the Hb concentration repeatedly over- and undershoots a target value or range, are a prevalent issue in the treatment of anemia (Schneider et al. 2010).

Anemia is diagnosed when hemoglobin levels fall below 11 g/dL in children aged 6–50 months, and below 11.5 g/dL in children older than 5 years. The severity of anemia is categorized as mild when hemoglobin levels are above 10 g/dL, moderate

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between 7 and 9.9 g/dL, and severe when levels are below 7 g/dL. Iron deficiency is indicated by Ferritinemia levels below 12 mg/L in children under 5 years old or below 15 mg/L in children over 5 years old (WHO 2011; Guerin et al. 2019). In the presence of infection or inflammation, the level of ferritinemia, which is the presence of iron storage protein in the blood, tends to increase by approximately 30%. This poses a risk of underestimating the prevalence of anemia caused by iron deficiency when there is concurrent inflammation (Balarajan et al. 2011). Anemia is frequently observed when a patient has chronic kidney disease (CKD), and it is linked to a lower quality of life for the patient. Moreover, it raises morbidity and death rates and quickens the rate of CKD progression (Cases et al. 2018). Anemia is primarily effect by a comparative defect of erythropoietin in CKD, primarily produced a hormone by the kidneys in adults (Haase 2013). There are several known causes of anemia in patients with ACLD (advanced chronic liver disease), including: First, gastrointestinal bleeding from gastro esophageal varies that results in acute or ongoing blood loss (Reiberger et al. 2017), gastrointestinal portal hypertension with stomach astral vascular ecstasies (Ripoll et al. 2011), Anemia can become worse in the presence of ACLD. Second, structural, and functional flaws in the lipid membrane of erythrocytes may result in the development of acanthocytes (spur cells), which have a short lifespan due to a higher sensitivity to splenic breakdown (Alexopoulou et al. 2014; Vassiliadis et al. 2010). Fourth, individuals with alcoholic liver disease have been linked to malnutrition and malabsorption, which results in vitamin B12 and folic acid deficits and macrocytosis and macrocytic anemia (Maruyama et al. 2001). An increased risk for hepatic encephalopathy is one of the clinical effects of anemia in ACLD, and this risk is linked to serum ammonia levels (Kalaitzakis et al. 2013). About 5% of the body's overall requirements for iron in adults come from dietary sources. This quantity is equivalent to iron loss, which primarily originates from the gastrointestinal tract. Old RBCs are broken down to provide the vast bulk (95%) of iron. Due to their rapid growth during the pediatric age range, children get about 30% of their iron from diet (Bhattacharya et al. 2016; Lönnerdal et al. 2015).

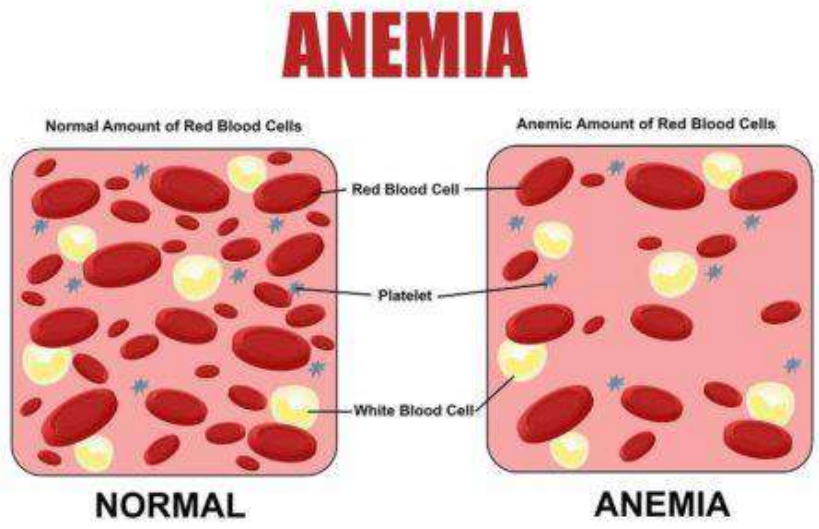

Figure 1.1 Difference between anemias with normal Hb concentration (Coyer SM 2005).

1.2 Background history:

Hünefeld discovered haemoglobin (Hb) by accident in 1840 in samples of earthworm blood sandwiched between two glass slides. He discovered tiny plate-like crystals in dried swine or human blood samples on occasion [Hünefeld F. L (1840), Giegé R 2013]. Hoppe-Seyler later termed these crystals "Haemoglobin" in 1864 [Hoppe-Seyler F 1926].

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Claude Bernard discovered its significance as an oxygen carrier in 1870 [Blumenthal I. 2001]. Hb is an iron-containing oxygen-transport metalloprotein present mostly in all vertebrate red blood cells (RBCs) [Anthea M et al 1993]. Haemoglobin (Hb) is a key protein involved in oxygen (O2) transfer. Red blood cells (RBCs) have the highest concentration of Hb and, due to their unique shape and plasticity, carry O2 to all tissues in the body at the ideal concentration. It was recently discovered that, in addition to RBCs, Hb is expressed by non erythroid cells such as epithelial cells of various origin [Saha, D et al. 2014].

Anemia is defined as a haemoglobin concentration below a certain threshold; the threshold varies depending on the age, gender, physiological status, smoking habits, and altitude of the group being tested. Anemia is defined by WHO as a haemoglobin concentration of 110 g/L at sea level in children under the age of five and pregnant women, and a haemoglobin concentration of 120 g/L in non-pregnant women. Anemia has been linked to an increased risk of maternal and infant mortality. Individuals and entire populations suffer from iron deficiency anemia, which has major ramifications for the economic and national development. Furthermore, the harmful effects of iron deficiency anemia on children's cognitive and physical development, as well as physical performance - specifically adult job productivity - are serious issues. Anemia is a worldwide issue that affects all countries [WHO 2014].

Iron deficiency is thought to be the most common cause of anaemia globally, but other nutritional deficiencies (including folate, vitamin B12 and vitamin A), acute and chronic inflammation, parasitic infections, and inherited or acquired disorders that affect haemoglobin synthesis, red blood cell production or red blood cell survival, can all cause anaemia. Haemoglobin concentration alone cannot be used to diagnose iron deficiency. However, the concentration of haemoglobin should be measured, even though not all anaemia is caused by iron deficiency. The prevalence of anaemia is an important health indicator and when it is used with other measurements of iron status the haemoglobin concentration can provide information about the severity of iron deficiency [WHO 2007].

1.3 Anemia in CKD Patients:

Anemia is most common complicated CKD, However, it remains uncertain what the ideal hemoglobin levels should be for individuals in different stages of chronic kidney disease. Anemia significantly increases the risk of complications and cardiovascular-related mortality in patients with this condition [Weiner DE et al. 2005]. Moreover, it amplifies illness and death rates and accelerates the progression of chronic kidney disease. Individuals with CKD often experience an ongoing state of inflammation, which is linked to a wide array of underlying factors [Gluba-Brzózka, A etal. 2020]. Individuals with CKD commonly experience a persistent inflammatory condition that is associated with a broad spectrum of underlying causes, including a heightened occurrence of infections, elevated proinflammatory cytokine levels, the uremic environment, the prevalent existence of arteriosclerosis, and various other factors [Malyszko, J et al. 2007]. Anemia among individuals with CKD is a complex phenomenon, involving a multitude of factors. These include persistent inflammation, insufficient production of erythropoietin, disruptions in iron metabolism, blood loss during hemodialysis treatments, unregulated hyperparathyroidism, scarcities in crucial nutrients like iron, folic acid, and vitamin B12, as well as the utilization of certain medications such as ACE inhibitors and the influence of uremic toxins [Căldăraru et al. 2017, Babitt et al. 2012, Zadrazil et al. 2015 and Bataille S et al. 2017]. In addition to actual iron deficiency, many CKD patients exhibit functional iron deficiency. This condition is marked by impaired release of iron from bodily reserves, hindering the fulfillment of erythropoiesis requirements. In this subset of patients, low serum transferrin saturation (indicative of circulating iron levels) and normal or elevated serum ferritin (reflecting body iron stores) have been demonstrated. The presence of secondary hyperparathyroidism, a common occurrence in CKD, further contributes to the progression of anemia and heightened resistance to erythropoietin [Weiss, G 2002]. Ultimately, hepcidin, a pivotal controller of the absorption of circulating iron, has been identified as playing a role in the origin of anemia within CKD [Wagner, M et al. 2013, Atkinson, M et al. 2015].

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Around 1.2 million individuals lost their lives due to chronic kidney disease (CKD) and consequent end-stage renal disease (ESRD) on a global scale in 2015. This marked a 31.7% rise from 2005. Notably, these conditions climbed the ranks to secure the top position as the primary cause of premature mortality between 1990 and 2005, extending through to 2015 [GBD 2015]. Identifying and addressing CKD at an early stage and focusing on prevention among individuals with elevated risk are tactics employed to alleviate the impact of the condition. Yet, given that a significant number of patients show no symptoms until CKD reaches advanced stages, the task of identifying suitable candidates for screening and pinpointing asymptomatic adults at higher risk has posed a considerable challenge [Qaseem A et al. 2013]. The influence of hemoglobin (Hb) levels at the lower end of the normal range and the presence of anemia on the emergence of end-stage renal disease (ESRD) is noteworthy. It should be noted that reduced Hb levels alone could potentially play a role in advancing CKD towards ESRD [Iseki K et al. 2003, Ishani A et al. 2006]. While a decrease in hemoglobin (Hb) levels and the onset of anemia might manifest as a result of CKD, even during its initial phases [KDIGO 2011]. Hemoglobin levels that fall within the lower normal range, for instance, 13–13.9 g/dL in males, have received limited consideration. Moreover, this aspect has been infrequently investigated, and it remains uncertain whether having low-normal hemoglobin levels or experiencing anemia heightens the likelihood of developing ESRD in individuals who possess normal kidney function, specifically those without albuminuria and possessing a normal glomerular filtration rate (GFR) [Yi, Sang-Wook et al. 2019].

1.3.1 Ineffective erythropoiesis of anemia:

Erythrocytes' ability to carry oxygen comes from heme, a crucial component that may bind to iron and transport oxygen to tissue through moving red blood cells (RBC). Because of this, mammalian systems have a close relationship between erythropoiesis and iron levels to maintain a healthy balance between iron uses for the production of new erythroblasts and iron recycling from senescent erythrocytes [Gupta, R et al. 2018]. In the bone marrow and spleen, erythropoiesis takes place in specific niches with a central nursing macrophage surrounded by erythroid cells at various stages of development [An X et al. 2011]. Erythropoiesis occurs in distinct niches with a central nursing macrophage and erythroid cells at various stages of development surrounding it in the bone marrow and spleen [Finch CA 1959]. An increase in erythron iron turnover and a decrease in RBC use have been found to be signs of inefficient erythropoiesis [Pootrakul P et al. 1989]. A total of 95% of the iron taken up by erythroid cells in the bone marrow was eventually found in circulating RBCs, demonstrating that under normal circumstances, 95% of red cell production is successful, whereas 5% is ineffective [Longo F et al. 2021] In normal people, the proportion of inefficient erythropoiesis was thought to be around 5% of total erythroid activity [Barosi G et al. 1978]. The rate of erythropoiesis may be greatly increased to boost oxygen supply to the tissue under situations of low oxygen delivery, such as hemolysis, high altitude, or acute anemia [Paulson et al. 2011]. In contrast, in cases where erythropoiesis is pathologically changed, such as -thalassemia, the erythrocytes' short lifespan and poor capacity to transport oxygen promote a harmful state of stressed erythropoiesis and favor the failure of effective RBC synthesis [Crielaard et al. 2014].

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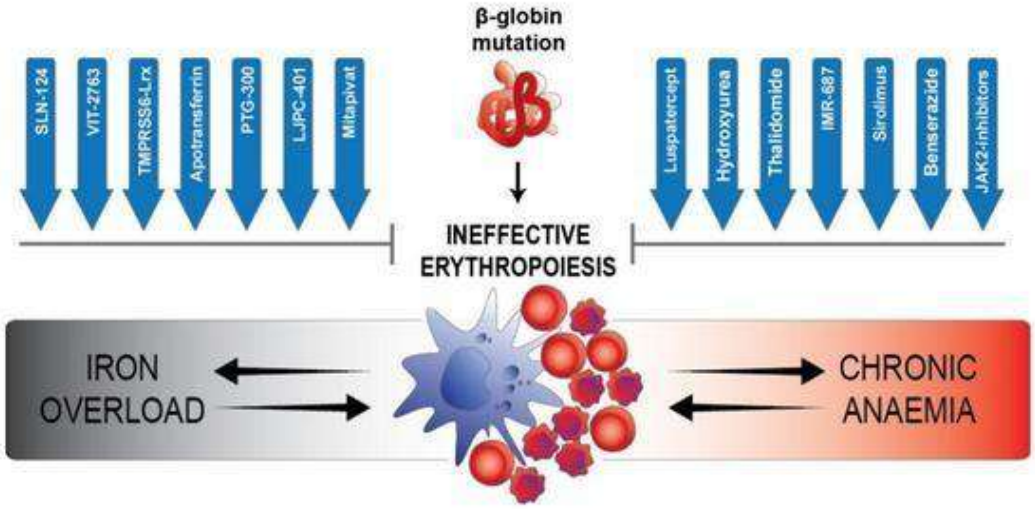

Figure 1.3 1 Ineffective erythropoiesis is a persistent state of stress erythropoiesis seen in some illnesses.

A central nursing macrophage is surrounded by erythroid cells that are at various stages of development during erythropoiesis, which takes place in specialized niches inside the bone marrow and the spleen [Sadahira Y et al.1990]. Ineffective erythropoiesis is a persistent state of stress erythropoiesis seen in some illnesses. An increase in erythroblast proliferation that fails to differentiate and give birth to enucleate RBCs, which results in anemia under inefficient erythropoiesis, characterizes the imbalance of erythroid proliferation and differentiation [Chow A et al. 2013].

1.3.2 Hemoglobin Concentration of anemia:

A low blood hemoglobin concentration is referred to as anemia. The World Health Organization (WHO) establishes the hemoglobin levels that constitute anemia, and they varies for men, women, and children [Darnton-Hill et al. 2005]. The World Health Organization (WHO) estimates that 1.62 billion people worldwide suffer from anemia, with low- and middle-income nations having the highest prevalence. Anemia is thought to affect 32% of African children under the age of five and 44% of expectant mothers [WHO 2011]. Anemia still affects 11% of children under the age of five, 16% of women who are reproductive age (WRA), and 22% of pregnant women in high-income nations. Low birth weight and an increased risk of maternal and perinatal death are linked to anemia during pregnancy [Stevens, G.A et al. 2011]. Anemia is a clinical illness that, physiologically, is brought on by a decrease in the mass of circulating red blood cells. It is frequently measured in terms of the hemoglobin (Hb) concentration of the entire blood [Lee GR 1999]. Age, sex, pregnancy, health state, as well as genetic and environmental factors, all influence Hb concentration [Whitehead et al. 2019, WHO 2017]. Normal Hb concentrations range from 170 to 210 g/L in newborns, dropping to roughly 100 g/L for infants aged 6 to 9 months during the first few months of life [Domoloff M et al. 2002]. prior to rising once again during infancy to roughly 110 g/L up to 59 months, 115 g/L up to 11 years, and 120 g/L for adult females and 130 g/L for adult males [Jorgensen et al. 2019]

1.4 Classification of Anemia in Hb LEVEL

Anemia can be categorized according to the underlying cause, such as higher red cell loss in comparison to decreased production. Anemia can be microcytic (caused, for example, by a lack of iron or thalassemia), normocytic (caused, for example, by inflammation), or macrocytic (caused, for example, by a lack of folate or vitamin B12, liver illness, myelodysplasia, or hypothyroidism) [WHO 2017].
Although it is of limited help in determining the cause of anemia, morphological characterization is typically more convenient to assist the diagnosis and indirectly the treatment in association with the laboratory findings and clinical evaluation. At the individual and societal levels, the severity of anemia

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can be categorized as a public health burden. Anemia in the two situations might range from mild to severe. Anemia is currently seen as a public health issue [WHO 2011].

Table 1. 1 Table 1 Classification of public health significance of anaemia in populations on the basis of prevalence estimated from blood levels of haemoglobin [WHO 2011].

Category of public health significance	Prevalence of anemia (%)
Severe	40 or higher
Moderate	20.0–39.9
Mild	5.0–19.9
Normal	4.9 or lower

Table 1. 2 Table 2Hemoglobin levels (g/L) to diagnose anemia at sea level [WHO 2011].

	Anemia			
Population, age	**No anemia**	**Mild**	**Moderate**	**Severe**
Children, 6–59 months	≥110	100–109	70–99	<70
Children, 5–11 years	≥115	110–114	80–109	<80
Children, 12–14 years	≥120	110–119	80–109	<80
Nonpregnant women, 15 years and above	≥120	110–119	80–109	<80
Pregnant women	≥110	100–109	70–99	<70
Men, 15 years of age and above	≥130	110–129	80–109	<80

The World Health Organization is revising its recommendations for the use and interpretation of Hb values to identify anemia in both individuals and populations [Garcia et al. 2019]. However, even the diagnosis of anemia with Hb measurement is a low cost, and more widely available in clinical settings to know the anemic status of the individual [Kerkhoff et al. 2015].

1.5 Causes of Anemia:

Nutrient shortages are one of the most common causes of true or extreme anemia. Frequently, there are several deficits present, and the clinical appearance may be worsened by accompanying infections, generally poor nutrition, or inherited diseases such hemoglobinopathies. The most prevalent anemias are megaloblastic anemia and iron-deficiency anemia. These anemias are more prevalent in women who do not take prenatal iron and folate supplements and who have poor diets. Hemolytic anemia and aplastic anemia are two additional, less frequent forms of acquired anemia during pregnancy. Additionally, anemias such thalassemia and sickle cell disease might affect the mother's and fetus's health. These deficiencies are frequently many, and the clinical presentation may be more severe by accompanying infections, generally poor diet, or inherited diseases such hemoglobinopathies. (BAKER S J 1983, WILLIAMS et al. 1992). A lack of iron is the cause of about 75% of all anemia's identified during pregnancy. Characteristic hypochromic, microcytic erythrocytes on the peripheral blood smear indicate a significant iron shortage. It is important to take into account additional, albeit uncommon, causes of hypochromic anemia's, such as hemoglobinopathies, inflammatory processes, chemical toxicity, malignancy, and pyridoxine-responsive anemia.

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The megaloblastic anemias of pregnancy caused by folic acid insufficiency and, to a lesser extent, by vitamin B12 deficiency, make up the majority of the remaining cases of anemia in pregnancy other than the iron-deficiency variety. Humans rarely have anemia brought on by a lack of other vitamins or minerals [COOK J.D. 1983].

1.6 Objectives:

Our thesis objective is currently determining data of anemic children's level of hemoglobin diagnosed in the population of Shaheed Benazirabad.

- To assess the parameter HB, RBCs, HCT, MCV and MCHC analysis blood concentration in our body.
- To examines the hemoglobin level parameters identified the Hb concentration may be high and low and how patients they can survive with the treatment and cure disease

Chapter 2 Literature View

2.1 Literature View

Anemia iron deficiency and Anemia is known to significantly degrade functioning, increase mobility (life space), and increase mortality (in older male/ female), decrease quality of life and may have an impact on treatments costs. Anemia is a potentially preventable condition with significant medical consequences. It has serious consequences for children's growth and development in their early years of life (Nambiema et al. 2019). Anemia affects 1.62 billion people worldwide (Iglesias et al. 2019). A haemoglobin level of less than 11.0 g/dl is considered to indicate anaemia, which is most prevalent in children aged 6 to 59 months and pregnant women (Dutta et al. 2020).

Associated with childhood anemia has been prolonged growth, an increased risk of infections, and subpar cognitive and motor development (WHO 2017; PAHO 2009; Best et al. 2014). According to the age of the children, the prevalence of anemia there was substantial variation, with preschoolers having a larger proportion of anaemia than those in school-age children. Up to age 5, the growth rate and nutrient needs are considerable (Kliegman et al. 2016). Anemia due to iron deficiency is one of the most common types of malnutrition in the country, accounting for over 48% of anemic children (De Benoist et al. 2005) It is one of the leading causes of morbidity and mortality in children (Joshi PC 2012). Similarly, adult children groups serve with anemia produced by iron deficiency and malaria are at higher mortality risk (Hu et al. 2014; Black et al. 2016). Recently policy of public health promotes iron supplements or dietary products enhance prevention to child in general malnutrition with anemia during pregnancy (Ethiopia et al. 2018). Anemia iron deficiency is difficult to diagnosis that manage in the presence of acute or chronic inflammation (Grant et al. 2012). considered anemia is a public serve health problem if it is 40% higher prevalence, according to World Health Organization criteria. Furthermore, India falls into the severe category for both children and pregnant women, Significance of Anemia according to the WHO cclassification of Countries by Degree of Public Health (WHO 2015).

Anemia and other mild types of micronutrient deficiency can have negative effects on a person's health and ability to develop, while severe anemia is frequently linked to higher rates of maternal mortality, premature birth, low birth weight, and delayed child development. In addition, the cause of anemia burden is genetically hemoglobin abnormalities (WHO 2017; Allen et al. 2006).

Numerous factor due to contribute to anemia, targeted interventions cannot be implemented without first in the region of interest assessing determinants of anemia. There is some information about The Gambia's moms and young children's nutritional and micronutrient status, but these data are outdated at the national level, and there isn't a thorough analysis that evaluates both status macronutrient and micronutrient. Approximately 73% of children and 58% of non-pregnant women of reproductive age in 2013 after affected (Oh et al. 2020).

Anemia during pregnancy increased the risk of childhood anemia. A public health approach that priorities pre-conception maternal health would allow for improved maternal and child morbidity risk prevention (Wirawan et al. 2022). Pre-pregnancy intervention or iron supplementation Pre-conception iron storage increases in infants (Nguyen et al. 2016). Anemia as identified hemoglobin levels only about 6

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g/dl, is linked to a poor fertility. In the maternal side anemia included many complications of severe such as prematurity, spontaneous abortions, low birth weight, and fatal (micronutrients status development of chronic disease) deaths. Nevertheless, concentration of fetal hemoglobin does not appear by mild to moderate iron deficiency to be affected (Sifakis et al. 2000).

Patients may exhibit symptoms associated with other situation such as lack of blood or symptoms related to oxygen-carrying capacity are decreased such as weakness, fatigue, andbreathing difficulty. The evaluation involves an extensive physical examination, medical history, assessment of variables risk for underlying situations that assessment of mean corpuscular volume. Measured serum ferritin levels in anemia patients with normocytic or microcytic. The low-level serum ferritin in a anemia patient with normocytic or microcytic is correlated with iron deficiency anemia. The treatment focuses on the underlying cause. Patients with symptoms and serum haemoglobin levels of 8 g/dL or less may require a blood transfusion. Iron oral replacement therapy tried on patients with suspected iron deficiency anemia. Formulations may be Lower-dose as effective higher-dose while posing a adverse effects of lower risk. Most patients' haemoglobin levels return to normal within eight weeks of treatment. Parenteral iron infusion is only used for individuals who did not respond to or could not tolerate oral iron treatment (Brian et al. 2018)

The most Hemoglobin (Hb) concentration used to anemia diagnoses. Individual must be identified to diagnosis that requires individual treatment. Estimates of anemia prevalence at the population level are frequently used to motivate national nutrition policies or programs. As a result, the critical Hb measurement be sensitive, specific, accurate, and reproducible (Karakochuk et al. 2019). Aggressive methods for measuring Hb (requiring a blood sample) include hematology automated analyzers (Fujimoto et al. 1999), hemoglobinometer (its device to use the hemoglobin concentration) (Srivastava et al. 2015). Over the last decade, in clinical chemistry for advances diagnostic have resulted in the development of numerous new methods for assessing anemia (Barker et al. 2008). Hb concentration, hematocrit (or packed cell volume), or RBC concentration can all be used to determine it. These markers are counted manually or with the use of haematology analyzers or automated cell counters. Automated haematology analyzers are increasingly commonly utilized due to their user-friendliness and automated laboratory operations, calibration, and quality control assurance (Gulati et al. 1986; Mohandas et al. 1986). These analyzers give a comprehensive blood count with extra characteristics along with the counting and characterization of blood cells., such as mean corpuscular volume (MCV) for determining microcytic, normocytic, or macrocytic anemia, to aid in the identification of anemia subtypes (Karakochuk et al. 2019). For men, the normal haemoglobin ranges from 14 to 18 g per deciliter of blood, and for women, it is 12 to 16 g. Men's and women's typical haematocrits are 42-54% and 36-48%, respectively (Reid et al. 2004).

The low hemoglobin levels are defined Hypochromic microcytic anemia (HMA) included Hb parameter are mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) levels. In children iron deficiency anemia is the most common cause of macrocytic anemia disease (Mohammed et al. 2021). The severity of anemia was associated with low body weight and infants born via caesarean delivery, with no gender influence. The CBC indices of RBC count, HGB, MCV, and mean corpuscular hemoglobin (MCH) showed a significant difference (values 0.05) between iron deficiency infant populations (Amer et al. 2022). In initial state anemia is diagnostic test from complete blood count (CBC) machine in which differentiation of microcytic, normocytic, and macrocytic anemia commonly based on the mean corpuscular volume (Mantadakis et al. 2020).

While macrocytic anemia is uncommon, microcytic iron deficiency anemia (IDA) is a prevalent cause of childhood anemia. IDA in infants aged 6 to 12 months can be caused by consuming less iron than is recommended. The recommended daily iron intake for children is determined by their age and gender. The mentioned daily iron uptake in milligrams (mg) from birth to six months is 0.27, and this amount is increased to 11 (R T 2020). The most frequent reason is a diet with insufficient iron. Much more iron is required during times of rapid growth (Pagani

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et al. 2019). Iron-limited erythropoiesis is distinguished by a decrease in both reticulocyte HGB content and mean corpuscular volume, but not in HGB or haematocrit (HCT) (Macdougall 2011; Huang et al. 2019). Prior to the last stage of anemia, erythrocyte parameters including MCV and MCH are disrupted. Which is accompanied by a drop in Hb levels below the limit? As previously suggested, a decrease in MCV and MCH in this study could indicate a deficiency in micronutrients such as iron and vitamins (Ramzan et al. 2009). It is outdated assumption, it may be misleading to suppose that anemia always indicates a haemoglobin deficiency, which could lead to tests and treatments that are more appropriate for addressing "haemoglobin deficiency" than "plasma volume excess." (Otto et al. 2017).

N/10 HC1 was dispensed up to the graded dilution tube 20% mark using an ordinary pipette. The heparinized blood was pipetted into the dilution tube with a hemoglobin pipette filled to 0.02 ml. The stirrer was used to mix the blood in the dilution tube contain HCL solution. It was diluted by adding distilled water and standard tubes were the same colour. The hemoglobin level was recorded as g/dl of blood (Wintrobe et al. 1967).

2.2 Classification of Erythrocytes morphology:

Red blood cell morphology is another system for classifying anemia. There are three basic divisions within the morphologic classification system:

2.2.1 Microcytic anemia:

Anemia with a mean corpuscular volume (MCV) of less than 80 mcm3 in adults is referred to as microcytic anemia. The age of the patient, risk factors, and associated symptoms and signs should be taken into consideration when separating the congenital and acquired causes of microcytic anemia. Iron deficiency anemia is the most frequent cause of microcytic anemia; depending on the severity and other disorders of the patient, it can be treated with oral or intravenous iron. To reduce considerable morbidity and death, pregnant women and heart failure patients with iron deficiency anemia need to be treated differently [Rampon K 2023].

The following medical conditions cause microcytic anemia:

- Iron-deficiency anemia (IDA): This anemia is the most common cause of microcytic anemia.
- Thalassemias: These are blood disorders that affect your body's ability to make hemoglobin and red blood cells.
- Sideroblastic anemia: Sideroblastic anemia (SA) is a rare blood disorder that affects your bone marrow's ability to make normal red blood cells.
- Anemia of chronic disease: This form of anemia may develop if you have long-term illnesses that cause inflammation.
- Lead poisoning: Exposure to lead for a long time may cause microcytic anemia [Rampon K 2023].

2.2.1.1 Microcytic hypochromic anemia:

As the name implies, microcytic, hypochromic anemia is a kind of anemia in which the circulating RBCs are smaller than normal (microcytic) and have a diminished red color (hypochromic). Earlier in life women are more prone to hypochromic microcytic anemia because they lose blood with each menstrual cycle. Anemia affects around 41% of pregnant females and 30% of nonpregnant premenopausal females. Because of circulating testosterone levels, men are often resistant to anemia. Anemia affects 12.7% of adult males worldwide. Pre-school-aged children suffer the most from anemia after the female population due to a lack of iron in their primary diet. Human milk contains only 0.3 mg/L iron, which is insufficient [Budnevsky et al. 2017].

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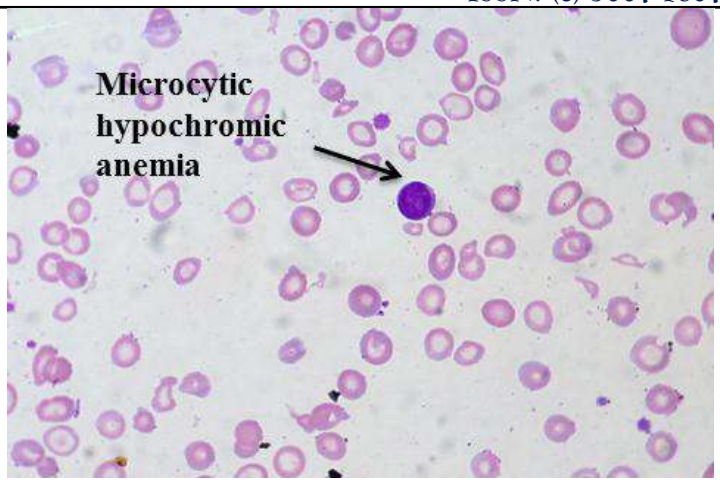

Figure 2.1 microcytic hypochromic anemias

Causes:

The mostly cause of microcytic anemia in children are thalassemia and iron anemia deficiency [Aydogan et al. 2019]. Iron insufficiency is the most common nutrient deficit in the world, and it is common in areas where nutrition is inadequate and socioeconomic level is low [Oski FA 1993]. Thalassemia is hereditary autosomal recessive hemolytic anemias caused by a reduction or absence of - or -globin chain production. Thalassemia is one of the most frequent single gene illnesses, with a high prevalence in the Mediterranean region as well as tropical and subtropical Asia and Africa. Thalassemia carriers make up 2.5% to 15% of the population in these areas [Canatan D 2014].

2.2.2 Macrocytic anemia:

Macrocytic anemia refers to macrocytosis (mean corpuscular volume (MCV) greater than 100 fL) in the setting of anemia (hemoglobin less than 12 g/dL or hematocrit (Hct) less than 36% in nonpregnant females, hemoglobin less than 11 g/dL in pregnant females, or hemoglobin less than 13 g/dL or Hct less than 41% in males). It is divided into two forms, megaloblastic (hypersegmented neutrophils) and non-megaloblastic. The megaloblastic form is due to impaired DNA synthesis from folate and/or vitamin B12 deficiencies, while the non-megaloblastic moiety occurs from multiple mechanisms [Lanier JB et al. 2018, Válka J et al. 2018]. Other times, macrocytosis is a sign that a patient is taking their medications as prescribed (for instance, methotrexate or zidovudine), and it only needs to be treated with supplements to prevent anemia [Nagao T et al. 2017, Green R et al. 2017].

The following medical conditions cause macrocytic anemia

- Folate deficiency anemia
- Anemia due to liver disease
- Hypothyroidism
- Alcoholism
- **Myelodysplastic syndrome**: This group of disorders happens when something's gone wrong with your bone marrow and it doesn't make healthy blood cells.
- **Alcohol use disorder**: Drinking excessive amounts of alcohol can keep your body from absorbing vitamin B12.
- **Hypothyroidism**: This condition affects your thyroid function and may be linked to macrocytic anemia [Moore C et al. 2022].

2.2.2.1 Associated with megablastic anemia:

Megaloblastic anemia (MA) refers to a type of macrocytic anemias distinguished by the presence of enormous red blood cell precursors known as megaloblasts in the bone marrow [Wickramasinghe SN 2006]. This syndrome is caused by faulty DNA synthesis, which prevents nuclear division. Cytoplasmic maturation, which is primarily determined by RNA and protein synthesis, is less hampered. This causes erythroblast nuclei and cytoplasm to mature asynchronously, explaining the megaloblasts' high size [Green R etal. 2017]. TRMA

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(thiamine-responsive megaloblastic anemia syndrome) is a rare inherited condition that has been discovered as a cause of megaloblastic anemia [Borgna-Pignatti C etal. 2009]. Microcytic, normocytic, or macrocytic anemia can result from clinical copper insufficiency. Myelodysplasia and megaloblastic anemia can be discovered with a bone marrow examination. Treatment with copper supplementation quickly reverses the disease's hematologic symptoms, though appearance may take longer [Wazir SM etal. 2017].

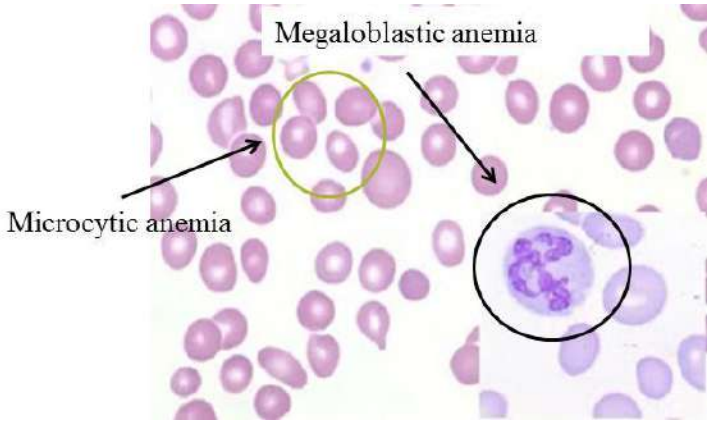

Figure 2.2 Microcytic anemia indicate the small size and irregular shapes in the RBCs

2.2.3 Normocytic normochromic anemia:

In contrast to other types of anemia, normocytic normochromic anemia has RBCs with average sizes and hemoglobin contents that are normally within normal ranges. Under a microscope, RBCs normally resemble normal cells, yet occasionally, differences in size and form may exist that balance one another out and produce average values that fall within the normal range. The most frequent causes of normocytic normochromic anemia are various chronic infections and systemic illnesses. The majority of normocytic anemias seem to result from poor RBC production [Stadler J et al. 2020]. A minority of the normochromic, normocytic anemias are caused by a main blood abnormality, while the majority are complications of other illnesses. Treatment of the underlying cause of anemia is the main component of management [Yilmaz et al. 2020].

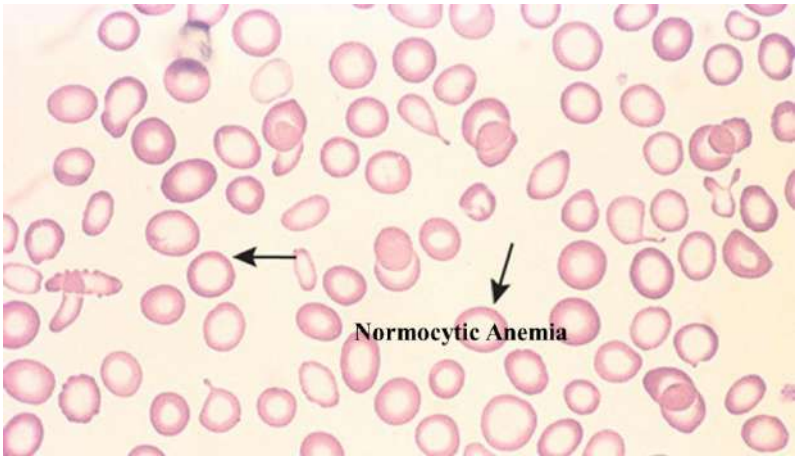

Figure 2.3 Normocytic and normochromic anemia indicate the normal size and color in RBCs

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2.3 Impact of low-normal Hb levels and mild anemia:

According to the results of the current study, men with Hb 13–13.9 g/dL had a more than doubled risk of end-stage renal disease (ESRD) compared to men with Hb 15 g/dL. Men with Hb 13-13.9 g/dL typically receive minimal attention in terms of their risk of CKD and ESRD because Hb 13 g/dL is considered anemic in men. Additionally, compared to their respective counterparts, mild anemia in men and women (11-11.9 g/dL and 11-12.9 g/dL, respectively) is linked to an elevated risk of ESRD by over two and four times, respectively. Proper therapy of males with Hb 13–13.9 g/dL and people with mild anemia at an early stage may be able to stop the development of CKD in the general population and stop the progression of CKD patients to ESRD [Mok Y et al. 2016].

2.4 Histopathology of hemolytic anemia:

Hemolytic anemia is characterized by the early breakdown of red blood cells, and it can be chronic or fatal. Any normocytic or macrocytic anemia should include it in the differential diagnosis. Hemolysis can happen intravascularly or extravascularly in the body [Phillips et al. 2018]. Due to low RBC deformability (i.e., the inability to alter shape enough to pass through the spleen), the primary extravascular mechanism is sequestration and phagocytosis. Antibody-mediated hemolysis can occur intravascularly or extravascularly and culminates in phagocytosis or complement-mediated destruction. Direct cellular destruction, fragmentation, and oxidation are examples of intravascular processes. Toxins, trauma, or lysis all induce direct cellular damage. Extrinsic stimuli cause shearing and rupture of RBCs, resulting in fragmentation hemolysis. When the cell's defensive defenses are overburdened, oxidative hemolysis ensues [Mentzer et al. 2018].

In clinical presentation, When a patient has acute jaundice or hematuria in the presence of anemia, hemolysis should be explored. Lymphadenopathy, hepatosplenomegaly, cholestasis, and choledocholithiasis are all symptoms of persistent hemolysis. Fatigue, dyspnea, hypotension, and tachycardia are other nonspecific symptoms [Phillips et al. 2018].

2.4.1 Iron deficiency anemia:

Iron deficiency anemia is the most prevalent and easily treated type of anemia. There is a growing recognition that iron deficiency can cause symptoms beyond from anemia and is linked to a number of disorders [DeLoughery, Thomas G 2017]. Iron deficiency anemia (IDA) is still one of the top five causes of years lived with disability in humans, and the primary cause in women [GBD 2017]. Although it has primarily been regarded as a public health issue affecting growing children, premenopausal and pregnant women, it is increasingly being recognized as a clinical condition that can affect patients presenting to various medical and surgical specialties, particularly those with chronic conditions and the elderly [Cappellini, M D et al. 2020].

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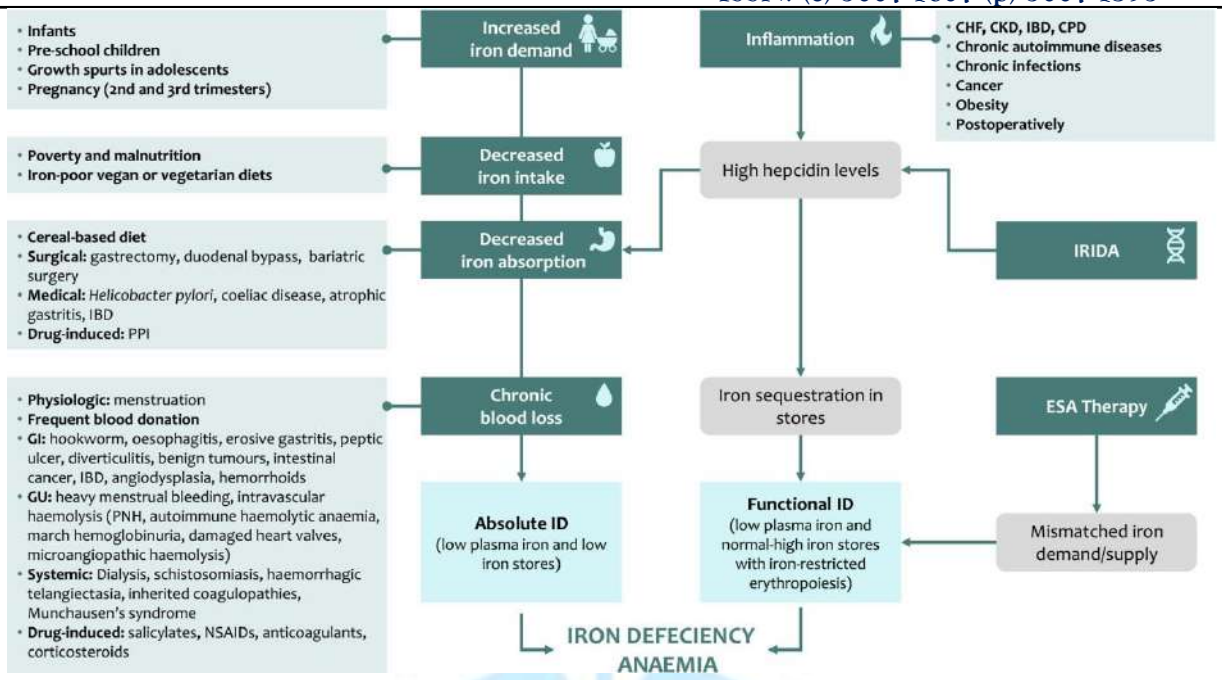

Figure 2.4 various etiologies of iron deficiency anemia; CHF, chronic heart failure; CKD, chronic kidney disease; CPD, chronic pulmonary disease; ESA, erythropoiesis-stimulating agents; IBD, inflammatory bowel disease; ID, iron deficiency; IRIDA, iron-refractory iron deficiency anemia; NSAIDs, No steroidal anti-inflammatory drugs; PNH, paroxysmal nocturnal haemoglobinuria; PPI, proton-pump inhibitors.

2.4.2 Folate deficiency (Vitamins B9 and B12):

The most common causes of megaloblastic anemia are vitamin B9 (folate) or vitamin B12 (cobalamin) deficiency. Folate can be found in green leafy vegetables, fruits, nuts, eggs, and meats. The normal folate reserves in the body range from 5 to 30 mg. The recommended daily limit varies depending on age, gender, and pregnancy status, although it is normally 400 g in adults and 600 g during pregnancy [Harrington DJ et al. 2018]. The three main cause of folate deficiency [Stabler SP 2013; Green R et al. 2017].

1. Reduced intake from folate-deficient foods (unusual in vitamin-fortified countries) and alcoholism
2. Reduced absorption due to small intestine illnesses that impact nutrient absorption, such as celiac disease, inflammatory bowel disease, and tropical spruce.
3. Increased demand due to pregnancy, hemolytic anemia, puberty, and eczema.

2.4.2.1 Vitamins B12 Deficiency:

Microorganisms create vitamin B12, which is found nearly exclusively in animal-derived foods. Vitamin B12 reserves in the body range from 3 to 5 mg, with an adult daily intake of 2.4 g suggested [Green R et al. 2017]. Vitamin B12 deficiency is less common than folate deficiency since body stores can remain for years due to effective enterohepatic recycling processes. Despite its rarity, dietary B12 deficiency can occur even in developed countries in devout vegans and vegetarians, as well as in breastfed newborns of women with vitamin B12 deficiency.

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Table 2.4.2.1 Vitamin B_{12} deficiency causes are mentioned in below [Daniel S et al. 2020].

Common causes (related to malabsorption)	Less common causes
Autoimmune gastritis (pernicious anemia)	Nutritional (strict vegans, breastfed infants of mothers with vitamin B_{12} deficiency)
Celiac disease	Nitrous oxide abuse
Inflammatory bowel disease	Diphyllobothrium latum infection
Surgical gastrectomy, gastric bypass, ileal resection	Pancreatic insufficiency
~	Drug effect (metformin, proton pump inhibitors)
~	Inherited disorders affecting intrinsic factor or the cubam receptor
~	Rare inherited disorder (eg, methylmalonic acidemia, transcobalamin II deficiency)

2.5 Abnormal erythrocytes cell physiology parameters:

2.5.1 RBCs (Red Blood Cells):

A human RBC has a life cycle of around 120 days. By this point, the cell is usually worn out and ruined. RBCs go through the spleen and liver, where they encounter specialized immune cells known as macrophages. When an RBC is depleted, macrophages recognize it and digest it in a process known as phagocytosis. The iron in haemoglobin is regenerated for usage in new blood cells during this process, and the hem molecule is destroyed, conjugated to bilirubin, and removed from the body. All of the other proteins in the cell are either recycled or destroyed. This process was formerly considered to occur solely in the spleen, but new research has revealed that it also happens in the bone marrow [Corrons et al. 2021].

2.5.2 HCT (Hematocrit):

The volume percentage (vol%) of red blood cells (RBCs) in blood, determined as part of a blood test, is known as the hematocrit (Ht or HCT) [Midline Plus 2020]. The amount and size of red blood cells determine the measurement. Males typically have 40.7-50.3% and females have 36.1-44.3%. Along with hemoglobin concentration, it is part of a person's full blood count findings. Anemia, or a decrease in the total number of red blood cells, is indicated by an abnormally low hematocrit, whereas polycythemia is indicated by an abnormally high hematocrit [Midline Plus 2019].

2.5.3 HB% (Hemoglobin level):

Anemia is defined as a decrease in hemoglobin (less than 13.5 g/dL in males and 12.0 g/dL in women) or hematocrit (less than 41.0% in men and 36.0% in women) or red blood cell (RBC) count. In everyday clinical practice, the words hemoglobin and hematocrit are more routinely employed than RBC count [Tas F et al. 2002].

Grading of anemia, according to the National Cancer Institute, is as follows:

1. Mild: Hemoglobin 10.0 g/dL to lower limit of normal
2. Moderate: Hemoglobin 8.0 to 10.0 g/dL
3. Severe: Hemoglobin 6.5 to 7.9 g/dL
4. Life-threatening: Hemoglobin less than 6.5 g/dL

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2.5.4 MCV (mean corpuscular volume):

The volume occupied by a single red blood cell is measured as mean corpuscular volume (MCV). In macrocytic anemia (vitamin B12 deficiency, folate deficit, alcohol usage), values are higher, whereas in microcytic anemia, values are lower.

The average hemoglobin concentration in RBCs is reflected in the mean corpuscular hemoglobin concentration. Spherocytosis has high levels, while thalassemia, iron deficiency, and macrocytic anemia have low values [Ray et al. 2020].

2.5.5 MCH (mean corpuscular hemoglobin):

MCH is the average red cell's hemoglobin (Hb) content (weight), or a reflection of hemoglobin mass in red cells. It is derived using the recorded Hb concentration (Hb) and red blood cell count (RBC) rather than measured directly [Vajpayee et al. 2011].

- MCH = Hb (in g/L)/RBC (in millions/µL) or
- MCH = [Hb (in g/dL)/RBC (in millions/µL)] x 10

The following are the reference ranges for mean corpuscular hemoglobin (MCH): [Pagana et al.2019].

- Adult/elderly/child: 27-31 pg
- Newborn: 32-34 pg

2.5.6 MCHC (mean corpuscular hemoglobin concentration):

Mean corpuscular hemoglobin concentration (MCHC) is a related number that is the average concentration of hemoglobin in a given volume of packed red blood cells [Vajpayee et al. 2011], or the ratio of hemoglobin mass to red cell volume [Perkins SL et al. 2009].

It is also not directly measured, but is computed using the Hb concentration (Hb) and the hematocrit (Hct):

- MCHC = Hb (in g/dL)/Hct (%)

The following are the reference ranges for mean corpuscular hemoglobin concentration (MCHC) [Pagana et al.2019].

32-36 g/dL (or 32-36%) for adults, elderly, and children

32-33 g/dL (or 32-33%) for newborns

Chapter 3 Material and method:

This study was conducted in the Department of Molecular Biology and Genetics, Shaheed Benazir Bhutto University Shaheed Benazir Abad (SBBUSBA) with the Collaboration of Pathology and Diagnostic Research Laboratory, at jhooly lal diagnostics laboratory and Al- Hyder diagnostic laboratory Nawabshah, Shaheed Benazir Abad from 1 August to 30 October in 2023. This study was based on lab procedure and all the total patients' 250 blood samples were collected and analyzed in HB% level for anemic patients and confirmed by performing test samples and microscopic abnormal erythrocytes cell are in appear on manual blood smear of anemic patients.

Samples were collected from the anemic patients by normal ranges compare to abnormal range according to the age groups, respectively. In these parameters shows different range of HB% level in anemic patients by RBCs range (4.7 to 6.1 Mil/cumm), HCT range (41 to 50 %), Hb level rang (14 to 18 g/dl), MCV range (80 to 100 fl), MCH range (27 to 31 pg) and MCHC range (32 to 36 G/dl). Data are divide into four age groups, 1-20, 21-49, 41-60 and 61-80 with gender differences ranges in these age groups shows the different ranges in the anemia affected patients including abnormal anemia affected erythrocytes cells are describe the three categories microcytic, macrocytic and normocytic and normochromic also shows as the size shapes and color in the inside red blood cells from patients samples.

The study was carried out with the permission of the Molecular Biology and Genetics Department, Shaheed Benazir Bhutto University, Shaheed Benazir Abad.

3.2 Methods and procedure for data analysis:

3.2.1 Sahli´s Method manual Hb level concentration:

Sahli's hemoglobinometer is a manual device that contains a haemoglobin tube, pipette, and stirrer, as well as a comparator. Hydrochloric acid converts haemoglobin to acid hematin, which is then diluted until the colour of the solution matches that of the comparator block. The clinician can then ascertain the haemoglobin concentration by reading from the calibration tube.

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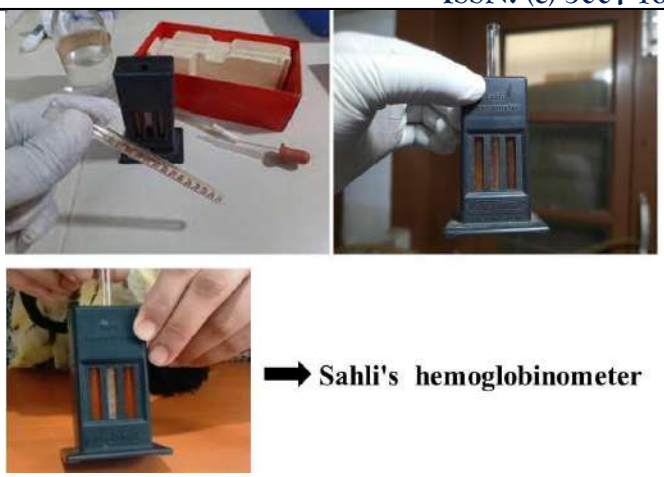

Figure 3.1 sahils hemoglobinometer is the device use to describe the mannully hb level performs

1. Ensure that the hemoglobinometer tubes and pipette are clean and dry.
2. Fill the hemoglobinometer tube with N/10 HCl up to its lowest mark i.e. 2 g% or 10% mark with the help of a dropper.
3. Take blood up to mark in the Sahli's pipette (20 µl). Wipe the extra blood outside the pipette and deliver it to N/10 HCl in the hemoglobin tube.
4. Mix and leave it for 10 minutes in order for a complete conversion of hemoglobin to hematin.
5. Add distilled water drop by drop and stir till color matches with the standard glass of the comparator.
6. Take the reading at lower meniscus, which directly gives the hemoglobin concentration in 100 ml of blood.

3.2.2. Automatic Method of CBC (complete blood count):

A full blood count (FBC), sometimes referred to as a complete blood count (CBC), is a series of laboratory tests used in medicine that reveal details about the cells in a patient's blood. The complete blood count (CBC) provides information on haemoglobin concentration, platelet, red blood cell, and white blood cell counts as well as the hematocrit (the volume % of red blood cells). A white blood cell differential, which counts the various types of white blood cells, may be included in addition to the red blood cell indices, which show the average size and haemoglobin concentration of red blood cells. An automated haematology analyzer typically performs the complete blood count (CBC), counting cells and gathering data on their size and shape. Haemoglobin concentration is determined, and red blood cell and haemoglobin values are used to compute red blood cell indices [Smock etal. 2018].

3.3 Demographic Measurements:

Each subject's HB% measured by measuring the distance of the cells in the slides displayed under the microscope. Physiological parameters including age (in years), sex and specimen identity.

3.3.1: Chemicals reagents:

Three chemical reagents used to determine the concentration of hemoglobin levels using slides of blood samples on a microscope. The chemicals regents are stain A, stain B and methanol. Stain A and Stain B is a field reagent consist of two parts thin and thick smear.

Stained (A) thick smears: A region measuring approximately 1.0–1.25 cm in diameter was created by extending a large drop of blood (10 l), with the corner of another microscope slide. Smear was fixed in methanol, stained, and allowed to air dry for at least an hour. Smear was studied using light microscopy with oil immersion at a 1000X magnification.

Stained (B) thin smears: A thin blood smear was created as normal using a little drop of blood (3-5 l)

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placed on a clean glass slide. Smear was fixed in methanol after a brief air dry.

Field staining chemical composition:

Stain A: It is a dark violet colour. It is consist of methylene blue and Azure 1 dissolved in phosphate buffer solution. Field stain A from the chemical's regents concentration Methylene blue (analytical grade) = 1.6 grams, Azur = 1 gram, Disodium dihydrogen phosphate anhydrous = 10 grams, Potassium dihydrogen phosphate anhydrous = 12.5 grams and the Distilled water = 1000 ml.

Stain B: It is an orange colour. It is consist of Eosin Y in buffer solution. Field stain B from the chemical's regents' concentration Eosin (yellow water soluble) powder = 2.0 grams, Disodium dehydrogenase phosphate anhydrous = 10-gram, Potassium dehydrogenase phosphate anhydrous = 12.5 grams and the Distilled water = 1000 mL.

Methanol: It is simple alcohol; they act as a fixative as well as a cellular stain. The best way of blood smear to Fix by dipping them in absolute methanol on the glass slides.

3.3.2: Interpretation of the cells in Field stain:

The resulting of field stains in the red blood cells will be appear pink in color. We will observe under the field stains Low pH, inadequate staining time, degraded stains, or excessive washing can result in excessively pink-staining blood films/slide. High pH, prolonged staining, or insufficient washing can result in excessively blue-staining blood films/slide.

We will observe variations in the size of RBCs, normal and abnormal amounts of hemoglobin levels, variations in shapes and colour during blood sampling on glass slide specimens. To determine the presence of an inclusions body in a blood sample.

RBCs count cells: RBCs were calculated by using the following formula: RBC (million/mm) = Cells counted/5 x 10x200. From the values of Hb and RBC count following useful erythrocyte indices was empirically calculated.

HCT: Hematocrit is the ratio of packages cells to total volume. To calculated the values of Hb multiply by 3.

Mean Corpuscular Volume (MCV): It expresses the average volume of the individual's RBC and is calculated from the formula such as MCV = Haematocrit X 10/R.B.C. MCV is expressed in femtoliter. A litter is slightly larger than a quart.

Mean Corpuscular Hemoglobin Concentration (MCHC): The average red blood cell haemoglobin concentration, or MCHC, is determined by applying a formula to determine the weight of the haemoglobin to the volume in which it is contained.

MCHC = Hemoglobin X 100/ Haematocrit

3.3.3 Field staining procedure:

1. Fill up two Coplin jars or wide-mouth bottles:
1. Field Stain A (Blue stain).
2. Field Stain B (Red stain).
2. Make a blood smear on a clean glass slide, and it is dried in the air.
3. Fix in methanol for one minute or get Spray 'Easyfix'.
4. Dry in the air.
5. Dip fixed smear to Field Stain B (Red Stain) for 5 to 6 seconds.
6. Wash in running tap water.
7. Dip smear into Field Stain A (Blue Stain) for 10 to 30 seconds (adjust it).
8. Wash in running tap water.
9. Dry at air and see under oil immersion objective.
10. The staining time may be adjusted.

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Figure 3.2 in the laboratory practice field staining research manually perform the slide blood smear through erythrocytes count abnormal cells individually blood samples at the Al-Hyder Research and Diagnostic laboratory Shaheed Benazirabad.

3.4 Analysis of data collection:

The blood samples will be analysed manually through the use of microscopes and three chemicals' stains under Stain A (dye consisting of methylene blue and azure 1 dissolved in phosphate buffer), stain B (dye composed of eosin Y in a buffer solution.) and methanol they have used to appear the slide of the blood sample that will be shows the hemoglobin level (HB), red blood cells (RBCs), haematocrit HCT, mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) these five parameters find out the means value of

HB% concentration in the anemic patients. We have use the MCV and MCHC formulas to analysis the HB% level in the patients, however HB% indicate the ranges of blood in the body.

3.4.1: Sample and data collection:

A total of 250 blood samples (infants to aged individuals) used in this study from anemia affected patients. Approximately 3 mL of blood will be collected in heparin tubes containing an anticoagulant solution and measured by using Sahli's hemoglobinometer method. The parameters such as Hemoglobin (HB; %), Haematocrit (HCT; %), Red blood cells (RBCs; %), Mean corpuscular volume (MCV; %) and mean corpuscular hemoglobin concentration (MCHC; %) used to measure the hemoglobin level in the patient. MCH and MCHC indicate the size and color of blood cells throughout our body.

3.4.2: Socio-demographic data:

The test was performed in 250 patients with a confirmed diagnosis of Hb level in anemic patients were included in this study. These all the anemic patients who were serve daily basis if iron deficiency, due to low level of blood in the body.

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3. 4.3: Manually perform and analysis research:

Microscopy Data analysis: We will observe in the blood smear slides shows many abnormal erythrocytes cells such as sickle cell, eliptocytes cell, tear drop cells, target cells, bur cells, acanthocytes cells, normocytic anemic cells, helmate cells, pencil cells. It is included hypochromic anemia such as moderate anemia, mild anemia and serve anemia it will appear low level of hemoglobin then normal hemoglobin level in the body. During abnormal erythrocytes cells we will see many different type of cells such as eliptocytes, target cells, tear drop cells, helmate cells, pencil cells, burr cells, acanthocytes cells, severely anemia, and mild anemia these cells are because different disease but we will discuss only Hb% level anemia affected cells. Anemia affected abnormal erythrocytes cells are distributed into three types of cells: 1. microcytic anemia characterized by color presences of small amount of RBCs in low MCV, 2. Macrocytic anemia characterized by large color presences of RBCs in high MCV and normocytic anemia characterized by same or normal in size, shape and color of RBCs.

Figure 3.3 After field staining of erythrocytes smear slides seen under the microscope, therefore many different types of cells research such as eliptocytes cells, tear drop cells, pencil cells, target cells, burr cells, helmate cells, acanthocytes cells and so on but we was observe only anemia Hb% level affected cells microscopy at the Al-Hyder Research and diagnostic laboratory Shaheed Benazirabad.

Chapter 4 Results

4.1 Demography of Study

Table 4.1 depicts the basic statistics for RBCs, HCT, Hb%, MCV, MCH and MCHC of female age groups at Shaheed Benazir Abad in 2023. Distribution frequency for these parameters was shown in Figure 4.1. The average value for RBCs ranged from 3.74 to 4.84 Mil/cumm, with minimum value showed by age group 61 to 80 and maximum by age group 21 to 40. The coefficient of variation was 63%. The average value for HCT ranged from 28.94 to 34.51 % with minimum HCT value showed by age group 0 to 20 and maximum by age group 41 to 60. The coefficient of variation was 26.36%. The average value for Hb% ranged from 9.67 to 11.48 g/dl. With minimum value showed by age group 61 to 80 and maximum by age group 41 to 60. The coefficient of variation was 26.6%. The average value for MCV ranged from 77.1 to 78.3 fL. With minimum value showed by age group 41 to 60 and maximum by age group 0 to 20. The coefficient of variation was 33.70%. The average value for MCH ranged from 25.6 to 26.7 pg. With minimum value showed by age group 41 to 60 and

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maximum by age group 21 to 40. The coefficient of variation was 31.47%. The average value for MCHC ranged from 33.2 to 33.5 g/dl. With minimum value showed by age group 41 to 60 and maximum by age group 0 to 20. The coefficient of variation was 31.70%.

Table 4.1 **Summary statistics for Hb % level of anemia of female age group in Shaheed Benazirabad**

Year		Age group					
		Referance Ranges	1 to 20 (mean ± SD)	21 to 40 (mean ± SD)	41 to 60 (mean ± SD)	61 to 80 (mean ± SD)	CV%
2023	RBCs	4.7 to 6.1 Mil/cumm	3.81 ± 1.09	4.84 ± 0.80	4.56 ± 3.94	3.74 ± 0.95	63%
	HCT	41 to 50 %	28.94 ± 8.32	32.65 ± 6.42	34.51 ± 6.96	29.06 ± 0.95	26.36%
	HB%	14 to 18 g/dl	9.69 ± 2.75	10.89 ± 2.10	11.48 ± 2.31	9.67 ± 2.79	26.66%
	MCV	80 to 100 fl	78.33 ± 31.17	80.11 ± 15.58	77.1 ± 2.31	77.13 ± 2.79	33.70%
	MCH	27 to 31 pg	25.9 ± 7.62	26.70 ± 5.19	25.64 ± 10.16	25.67 ± 2.59	31.47%
	MCHC	32 to 36 G/dl	33.5 ± 1.84	33.2 ± 0.18	33.26 ± 0.71	33.26 ± 0.08	31.70%

Table 4.2 depicts the basic statistics for RBCs, HCT, Hb%, MCV, MCH and MCHC of female age groups at Shaheed Benazir Abad in 2023. Distribution frequency for these parameters was shown in Figure 4.2. The average value for RBCs ranged from 3.14 to 6.04 Mil/cumm, with minimum value showed by age group 61 to 80 and maximum by age group 21 to 40. The coefficient of variation was 63%. The average value for HCT ranged from 30.48 to 37.16 % with minimum HCT value showed by age group 0 to 20 and maximum by age group 21 to 40. The coefficient of variation was 26.30%. The average value for Hb% ranged from 10.3 to 12.39 g/dl. With minimum value showed by age group 0 to 20 and maximum by age group 21 to 40. The coefficient of variation was 26.60%. The average value for MCV ranged from 74.95 to 113.1 fL. With minimum value showed by age group 0 to 20 and maximum by age group 61 to 80. The coefficient of variation was 33.75%. The average value for MCH ranged from 25.16 to 37.7 Pg. With minimum value showed by age group 0 to 20 and maximum by age group 61 to 80. The coefficient of variation was 31.60%. The average value for MCHC ranged from 33.3 to 33.84 g/dl. With minimum value showed by age group 41 to 60 and maximum by age group 0 to 20. The coefficient of variation was 42.75%.

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Table 4.2 Summary statistics for Hb % level of anemia of male age group in Shaheed Benazirabad

Year		Age group					
		Referance Ranges	1 to 20 (mean ± SD)	21 to 40 (mean ± SD)	41 to 60 (mean ± SD)	61 to 80 (mean ± SD)	CV%
2023	RBCs	4.7 to 6.1 Mil/cumm	4.1 ± 1.01	6.04 ± 7.15	4.27 ± 0.96	3.14 ± 0.61	63%
	HCT	41 to 50 %	30.48 ± 9.58	37.16 ± 7.93	33 ± 6.96	34.35 ± 3.48	26.30%
	HB%	14 to 18 g/dl	10.3 ± 3.37	12.39 ± 7.93	10.98 ± 6.96	11.45 ± 3.48	26.60%
	MCV	80 to 100 fl	74.95 ± 15.69	75.65 ± 25.52	80.22 ± 24.1	113.1 ± 24.8	33.75%
	MCH	27 to 31 pg	25.16 ± 5.01	28.6 ± 12.58	26.73 ± 7.99	37.7 ± 8.29	31.60%
	MCHC	32 to 36 G/dl	33.84 ± 4.39	33.34 ± 0.07	33.3 ± 0.22	33.34 ± 0.04	42.75%

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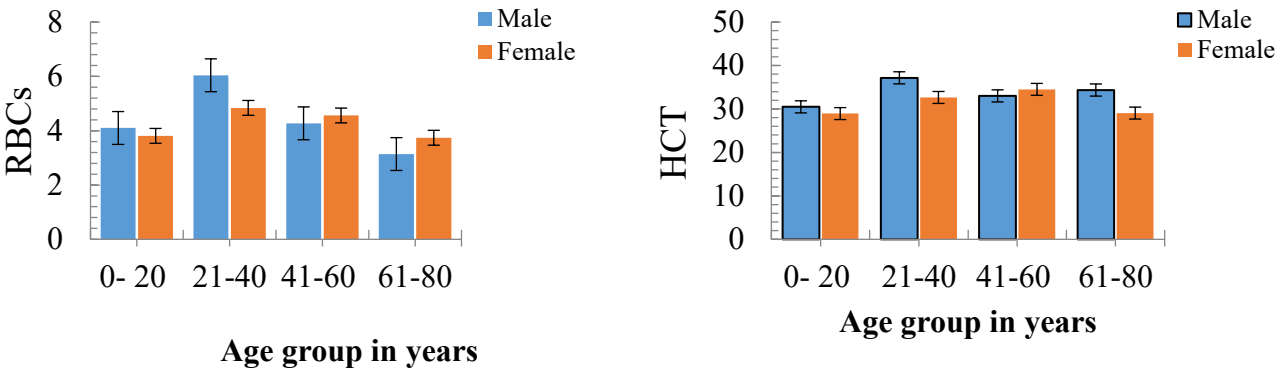

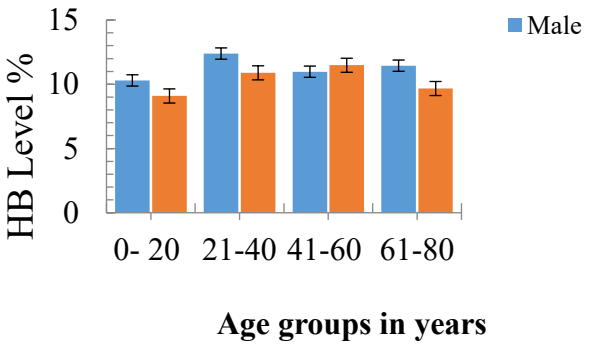

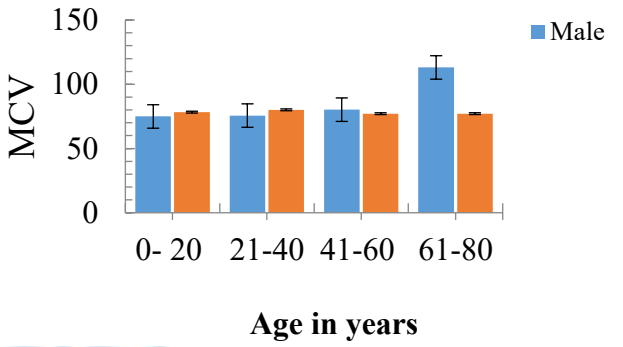

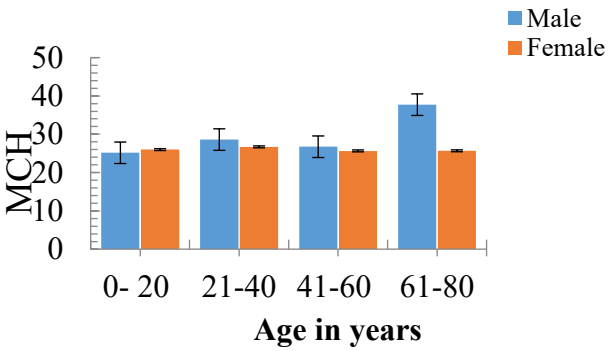

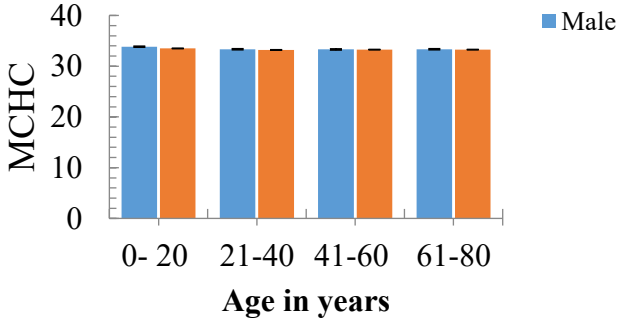

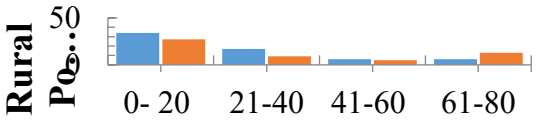

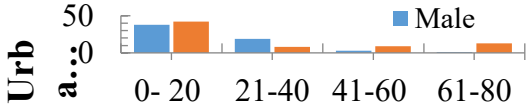

Figure 4.1 in this graph shows that the RBCs morphology average during the sex distribution with the parameter finding the average in age-wise groups 0-20, 21-40, 41-60 and 61-80, respectively. In the male and female individual parameters averages such as contain RBCs average in male > female with age group 21-40 years, HCT average in male (21-40 age in years) > female (41-60 age in years), Hb% average

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in male (21-40 age in years) > female (41-60 age in years), MCV average in male (61-80 age in years) > female (21-40 age in years), MCH average in male (61-80 age in years) > female (21-40 age in years), and MCHC average male and female in equal with the standard error devotion range.

Figure 4.2 in this graph shows that the average of the Hb% level anemia affected patients for population of Shaheed Benazirabad; area distributed the rural and urban with sex (male & female) and with the age groups in years also included the anemia patients. In the area distribution of rural patients in the average of males are greater than female in different individual age groups are mention, in urban patients in the average of female are greater than male in different individual age groups.

4.2 Abnormal Erythrocytes cell identification during microscopy research:

During the abnormal erythrocytes cells microscopy, we was seen many different types of cell through blood smear/slides such as Eliptocytes Cells, Pencil Cells, Target Cells, Tear Drops Cells, Burr Cells, Sickle Cell, Stomatocytes, Acanthocytes, Helmate Cells. Therefore we was research on anemia affected cells are separated from these cells morphology, in the anemia affected cells are as eliptocytes cells, tear drop cells, pencil cell, sickle cells and target cells.

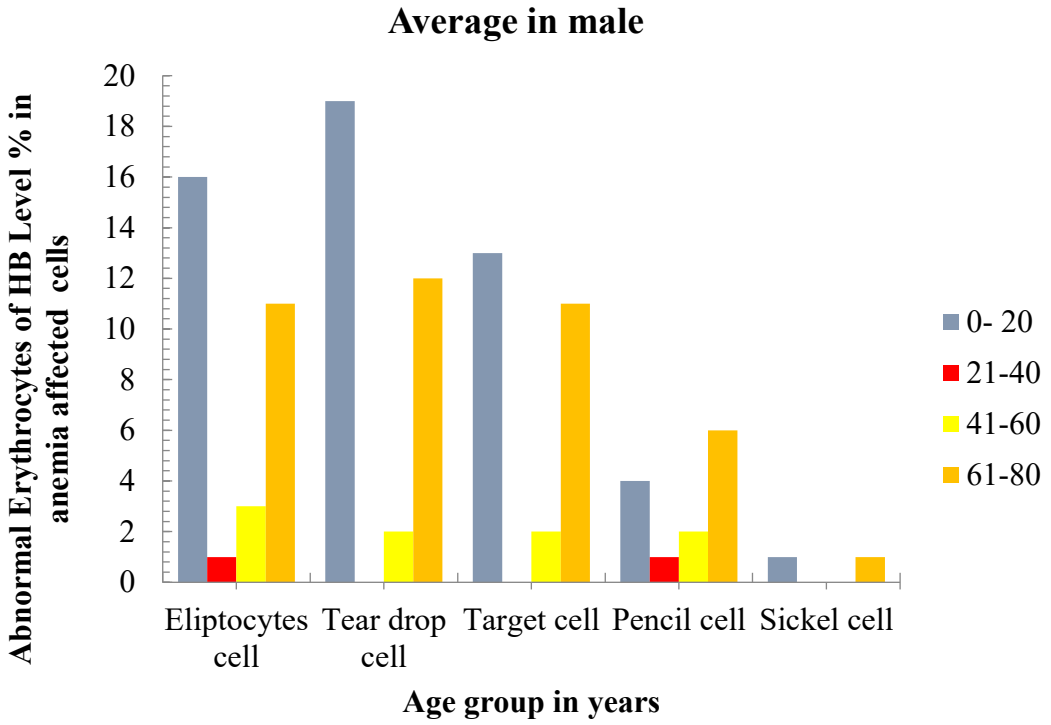

Figure 4.3.1 **in this graph show that the abnormal cells morphology of the average in male with the individual age group in years, in the Hb% level in anemia affected cells for the eliptocytes, tear drop cell, target cell, pencil cell and sickle cell are shown in different age group average are high but some abnormal cells average cannot appear in age groups because it's so rare in male age groups.**

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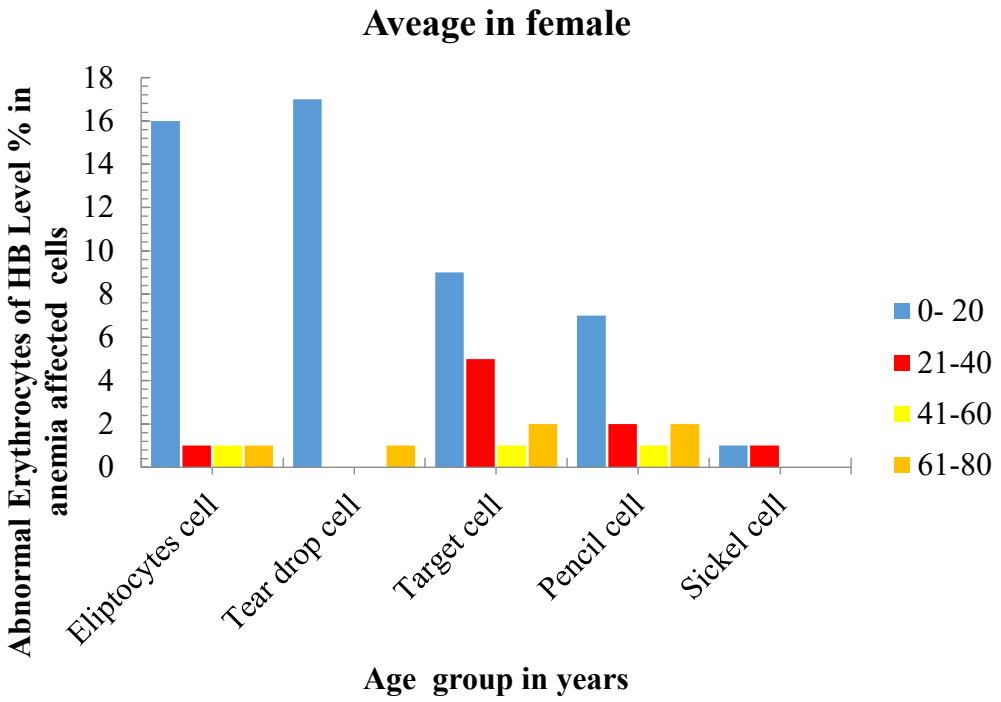
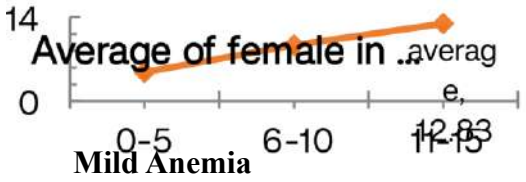


Figure 4.3.2 in this graph show that the abnormal cells morphology of the average in female with the individual age group in years, in the Hb% level in anemia affected cells for the eliptocytes, tear drop cell, target cell, pencil cell and sickle cell are shown in different age group average are high but some abnormal cells average cannot appear in age groups because it's so rare in female age groups.



Mild Anemia

Sev

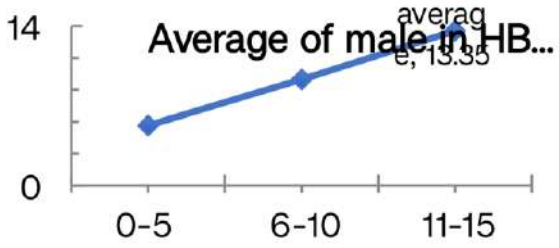


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Figure 4.4 in this graph lines shows that the average of male and female from Hb% level ranges low to high due to different individual ranges indicate the anemic patient and normal patients. In case 0-5 Hb% ranges shows severely anemia due to cause of thalassemia and sickle cell anemia in children with any age groups, 6-10 Hb% ranges shows mild anemia due to cause fatigue, iron deficiency and vitamin B9 OR B10 deficiency and 11-15 or above Hb% ranges show the normal population child to adults. In the average of male (13.35) greater than average of female (12.83).

Table 4.3 **Regarding mention the normal v/s affected Hb % level ranges for age wise respectively**

Age	Female range (g/dL)	Male range (g/dL)
0–30 days	13.4–19.9	13.4–19.9
31–60 days	10.7–17.1	10.7–17.1
2–3 months	9.0–14.1	9.0–14.1
3–6 months	9.5–14.1	9.5–14.1
6–12 months	11.3–14.1	11.3–14.1
1–5 years	10.9–15.0	10.9–15.0
5–11 years	11.9–15.0	11.9–15.0
11–18 years	11.9–15.0	12.7–17.7

Chapter 5 Discussion:

Anemia has recently been linked to poorer survival in a range of patients, according to growing evidence [Cordella et al. 2011; Finkelmeier et al. 2014; Zhang F et al. 2014]. Common anemia symptoms are non-specific and can resemble the features of the normal aging process, leaving patients undetected. Anemia is frequently a symptom of underlying co-morbid disorders in the elderly, which can impair quality of life. The anemia pattern can indicate the underlying disease etiology. As a result, in the older population, early detection of anemia, including morphological pattern, is crucial. Anemia of chronic disease (ACD) is the most common cause of elderly anemia (30-45%), which is caused by chronic infections and inflammatory disorders such as tuberculosis, infective endocarditis, chronic urinary tract infections, osteoarthritis, and rheumatoid arthritis. Approximately two-thirds of these instances have normocytic-normochromic anemia, while one-third have microcytic anemia. ACD is followed by anemia from dietary deficiencies such as iron, vitamin B-12, and zinc [Joosten E et al. 1992]. Iron deficiency anemia (IDA) can be established by serum ferritin, total iron binding capacity (TIBC), and serum iron measurements in conjunction with a microcytic image on a peripheral blood smear. IDA is frequently related with stomach or colon cancer in the elderly population. MDS and vitamin B12 and folic acid deficiency were frequently associated with macrocytic anemia. The presence of immature white cells, nucleated red blood cells, and pancytopenia may indicate underlying MDS, necessitating a bone marrow examination. Another prominent cause of geriatric anemia is chronic kidney disease (CKD), which should be suspected in patients with normocytic-normochromic anemia and a glomerular filtration rate (GFR) of 60 ml/min [Choukimath et al. 2019; Jain V 2019]. Anemia is a common hematologic problem in patients, with the main indication being abnormal red blood cell parameters such as Hb, HCT, and MCV [Birgegard et al. 2005]. RBCs play an important part in whole blood function, namely

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oxygen transfer from the lungs to tissues for cell respiration. Different parameters in haematology are used to characterize RBCs: (a) their quantity, or the number of cells per litre of blood; (b) their size, or mean cellular volume (MCV); and (c) their haemoglobin content, or mean cellular haemoglobin (MCH) and mean cellular concentration of haemoglobin (MCCH). MCV, MCH, and MCCH are RBC indicators that are critical for morphologically classifying anemias as microcytic (low MCV), macrocytic (high MCV), and normocytic (normal MCV). However, in order to comprehend the physiopathology of hereditary hemolytic anemias (HHA) caused by RBC abnormalities, the most crucial RBC parameter is the shape of the RBCs [Vives et al. 2019]. This is not a static situation, and the morphology of circulating RBCs changes constantly under normal and pathological settings, making diagnosis easier. Scanning electronic microscopy (SEM) has been a useful complementary tool for exploring RBC shape for many years because a three-dimensional image has allowed a reinterpretation of the conventional morphology observation of RBCs by MGG-stained blood smears, where they are seen as squashed or flattened cells on extension [Berga L et al. 1987].

However, because of enormous advances in precision and accuracy, as well as the detection of numerous aberrant red cells by current hematology analyzers, automated results such as mildly hypochromic microcytic red cells and slightly decreased platelets can be autoreleased. Depending on the laboratory technique, a qualifying note, such as'microcytosis, consider iron deficiency and/or thalassemia', can be added to the results. This improvement allows technologists to focus more on major abnormalities, reducing overall workload and operating costs, decreasing turnaround time, and increasing laboratory efficiency. The capacity to microscopically verify or grade other aberrant RBC-M and use technology to aid in that process is essentially subjective and hence subject to change as it is influenced by competence [Constantino 2015].

Currently, there are two systems or methods of grading RBC-M [Gulati G. 2009; O'Connor BH 1984]. While some laboratories report or grade the degree of morphologic abnormalities numerically (plus) from 1+ to 4+, others used descriptive terms, such as slight (few), moderate, or marked, and/or 'rare' or 'occasional'. At times, others just report the abnormal morphologic findings as 'present'. Tables 1 and 2 show the condensed reference guide for grading abnormal RBC-M and the conditions associated with different grade levels, respectively. These tables are handy and can be easily used as a reference guide when performing microscopy.

Table 5.1 Table 5.1 1**Reference guide for grading red blood cell morphology [Constantino BT 2009].**

Red blood cells (cell type)	**Normal (nonspecific)**	**1+ (%) (Slight/few)**	**2+ (%) (Moderate)**	**3+ (%) (Marked)**
Hypochromasia		5–15	16–40	>40
(MCH – pg)	27–34 pg	22–26	18–21	<18
Polychromasia		3–5	6–20	>20
Microcytes (MCV – fL)	80–99 fL	70–79	60–69	<60
Macrocytes (MCV – fL)	80–99 fL	100–110	111–125	>125
Elliptocytes/Ovalocytes	~	6–20	21–50	>50
Target cells	~	5–10	11–25	>25
Acanthocytes	~	1–10	11–30	>30

pg, pictogram; fL, femtoliter, Teardrop cells accompanied by NRBCs can be reported even <4%.

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Table 5.2 Table 5.2 1**Conditions associated with abnormal RBC morphology based on their grading [Ford J 2013].**

Cell type	Slightly	Moderate
Elliptocytes/Ovalocytes	Megaloblastic anemia Severe iron deficiency anemia Sickle cell anemia Hypersplenic state Metastatic carcinoma Sideroblastic state Thalassemia trait	Hereditary pyropoikilocytosis Myelofibrosis Hemoglobin C trait South East Asian ovalocytosis
Target cell	Newborn/Premature infants Thalassemia minor Postsplenectomy Severe iron deficiency	A-C and A-S trait Thalassemia major Sickle cell anemia Sickle-thalassemia
Acanthocytes	Newborn/Old age Severe burns Sideroblastic anemia Enzymes deficiencies Anorexia nervosa/starvation Vitamin E deficiency Hypothyroidism	Renal disease Postsplenectomy Neonatal or acquired hepatitis Uremia McLeod phenotype Myxedema

It should be noted that the grading level of various ailments can range from mild to moderate, and even severe, depending on the severity, intensity, and length of the disorders.

The interpretation of the reference guide (Table 1) shown for illustration of the grading system of RBC-M is self-explanatory. This simplified reference guide is presented in a two-system or three-graded format: 1+ or slight, 2+ or moderate, and 3+ or marked. Notice there is no 4+, 'rare', or 'occasional'. Although the grading of abnormal results looks simple, the grading itself is complicated as it requires experience and expertise to identify and distinguish many abnormal cells.

More information on some morphological diseases, such as pencil cells, Howell-Jolly bodies, teardrops, agglutination, microcytic, sickle cells, and others, as well as how to analyze and grade abnormal RBC-M and their causes of production, can be found elsewhere [Bell A 1986]. Although haemoglobin levels do not directly alter cell shape, hypochromic anaemic cells have lower haemoglobin levels than normochromic cells. As the haemoglobin level drops, the center pallor zone expands, indicating that the cell has become hypochromic. The core pallor area and transparency of anaemic erythrocytes increase as hemoglobin levels fall. In other words, cells with a big central pallor area darken more than normal cells [Sirsendu, et al. 2013].

Chapter 6 Conclusion and future work:

In conclusion, with the advancement of age there is an low to high level of Hb% ranges including RBCs, HCT, HB, MCV, MCH and MCHC in male than female groups. Moreover, a significant of abnormal erythrocytes cells affects that the hemoglobin level in the anemic patients. These blood parameters assessments are used for several routine analyses and

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add up values in the proper diagnosis of CKD, and also used in the population of the SBA district.

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