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Pattern of Peripheral Blood Film Findings in a Tertiary Care Centre in Punjab – A Study of 10,000 Cases.

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ABSTRACT

Peripheral blood examination has been a window for hematological assessments since decades. Blood smear is often used to categorize and/or identify conditions that affect one or more types of blood cells. Examples include anemias, various infections, myeloproliferative disorders, thrombocytopenias and leukemias. To study the prevalence of various hematological conditions, sex wise distribution of various types of anemias. This was a cross-sectional study of 6 months duration conducted in a tertiary care hospital in punjab. Patients from all regions of punjab came to our hospital. They were categorized according to their age, sex and economic status. Ten thousands (10000) patients with hemoglobin less than 11 g/dl were identified. Normal study of pbf was observed in 18.9% while prevalence of anemia was 35.3%. It was more in females, being 70.1%, which included 48.7% of mild, 19.9% of moderate and 1.5% of severe anemia cases. Prevalence of anemia in males was 53.2%, with 34.3% mild, 17.7% moderate and 1.2% severe anemia. Incidence of neutrophilic leukocytosis with toxic granules was observed in 20.4%. It was more seen in females than males, being 52.5% and 47.5% respectively. Prevalence of pancytopenia, thrombocytopenia and leukaemia was 7.57%, 11.5% and 6.3% respectively. In a resource limited country like india, peripheral blood smear examination is a simple inexpensive, yet a valuable tool which helps in diagnosing various hematological disorders and guides for further management.

Keywords: Anemia, Pancytopenia, Thrombocytopenia, Myeloproliferative neoplasms, Leukaemia

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INTRODUCTION

Peripheral blood examination has been a window for hematological ongoing since decades. Analyzing blood films routinely has facilitated interpretation of various hematological disorders and has been a major diagnostic tool especially for etiopathological work up of anemias. A blood smear is often used to categorize and/or identify conditions that affect one or more type of blood cells and to monitor individuals undergoing treatment for these conditions. There are many diseases, disorders, and deficiencies that can affect the number and type of blood cells produced, their function, and their lifespan.

Examples include anemia, various infections, myeloproliferative neoplasms, bone marrow disorders, and leukemia. Usually, only normal, mature or nearly mature cells are released into the bloodstream, but certain circumstances can induce the bone marrow to release immature and/or abnormal cells into the circulation. When a significant number or type of abnormal cells are present, it suggests a disease or condition and prompts a health practitioner to do further testing.

Definition of anemia

Anemia was defined according to the WHO criteria as hemoglobin concentration lower than 12 g/dl in women and 13 g/dl in men. Along with most grading classification systems, mild grade anemia was defined as a hemoglobin concentration between 10.0 and 11.9 g/dl in women and between 10.0 and 12.9 g/dl in men, moderate anemia was defined as hemoglobin level of 7-9.9 g/dl and severe anemia was defined as hemoglobin levels less than 7 g/dl for both males and females respectively. For children 6 months to 5 years of age, anemia is defined as a Hb level < 11g/dl, children 5–11 years of age Hb< 11.5 g/dl, adults males Hb< 13 g/dl; non-pregnant females Hb< 12g/dl, and pregnant females Hb< 11g/dl⁽²⁾. Severe anemia is defined as Hb< 7.0 g/dl [1].

Neutrophilic Leucocytosis

Acute infections and inflammatory conditions usually show elevated counts of leukocytes. Leucocyte count more than 15000 cells/ul were considered leukocytosis. Already confirmed cases of malignancy/ leukaemia were excluded from this group. Morphological examination of neutrophils was done under magnifications of 400x and 1000x. Minimum of 50 neutrophils were counted per slide and an average impression was obtained. Neutrophils with toxic granules were defined by the presence of dark blue to purple colored granules in the cytoplasm. The most common tissue response in acute inflammation is dominated by neutrophils[2].

Thrombocytopenia

Thrombocytopenia (platelet count < 150,000/ μ l) is a common finding in peripheral blood film. Platelet count of 1,00,000 to 1,50,000/ul, 50000 to 1,00,000/ul and <50000/ul were classified as mild, moderate and severe thrombocytopenia respectively. Thrombocytopenia can also occur in association with anemia, infections and other myeloproliferative disorders/ leukaemia. Prolonged thrombocytopenia and absence of relative increase in the platelet count was also associated with a greater risk of mortality and thus require proper diagnosis.

Pancytopenia

Pancytopenia is the simultaneous presence of anemia, leucopenia and thrombocytopenia. Therefore it exists when hemoglobin (Hb) is less than 13.5g/dl in males or 11.5g/dl in females; leucocyte count is less than 4×10^9 /l and platelet count is less than 150×10^9 /l [3]. Pancytopenia was labeled as severe if patient had two or more of the following: Hb<7 gm%, ANC< 0.5×10^9 /l, and platelet count < 1.5×10^9 /l Particular emphasis was paid to bleeding tendencies like epistaxis, hematuria and hemetemesis.

Leukemia

A hematological malignancy arises when something goes wrong in the regulation of the division or the life span of a blood cell or its precursor. It is characterized by widespread, rapid and disorderly proliferation of

leukocytes and their precursors and by the presence of immature leukocytes in blood often in very large numbers. Leukemia is one of the most frequently occurring cancers in all races or ethnicities with relative proportion varying between 25-40%. Percentage of blast cells, red blood cell indices, white blood cell indices, number of platelets and the amount of hemoglobin was determined according to standard laboratory procedures. Slides were prepared with PBF (Peripheral Blood Film) by using 'Leishman stain' to find out the blast cells morphology in peripheral blood sample of all patients of blood cancer. Diagnosis of leukemia was done by 20% blast criteria and myeloperoxidase stain was used to distinguish between AML & ALL. [4]

MATERIAL AND METHODS

This was a cross-sectional study of 6 months duration conducted in a tertiary care hospital in Punjab. Patients from all regions of Punjab including Majha, Doaba and Malwa came to our hospital. Most of them were from Malwa region. They were categorized according to their age, sex and economic status. Socioeconomic status was based on total family income per month. In 1000 patients hemoglobin values was lower than 11 g/dl, with chief complaints, along with clinical correlation were identified. It was carried out with the objectives of standardization and grading of abnormal red blood cell, white blood cell and platelets morphology and number in peripheral blood smear.

Peripheral blood smears were prepared and stained with Leishman stain. Also included in study were peripheral blood films suggesting of neutrophilic leukocytosis, pancytopenia, various myeloproliferative disorders and leukaemias and thrombocytopenia of various causes. History of consanguinity, drug intake, especially anticonvulsant (sodium valporate), antibiotics (sulfa group), anti-malarial and anti-tuberculous agents, and recent infections was enquired about.

OBSERVATIONS AND RESULTS

Anemia and iron deficiency anemia are often used inter changeably. There are however mild to moderate forms of iron deficiency in which the host is not yet anemic, but tissues are functionally iron deficient. In addition, iron deficiency accounts for most of the anemia that occurs in underprivileged environments, multiple other causes exist independently or co-existent with this micro-nutrient deficiency. India is facing a grave public health problem, with the prevalence of anemia in India being > 40%. Anemia is an indicator of poor nutrition and poor health with major consequences for the human health, as well as for the social and economic development. Prevalence of anemia in our study was 35.3% of which 57.5% were females and 42.5% males were. It was more in females, being 70.1%, which included 48.7% of mild, 19.9% of moderate and 1.5% of severe anemia cases. Prevalence of anemia in males was 53.2%, with 34.3% suffering from mild, 17.7% from moderate and 1.2% from severe anemia. Prevalence of anemia was more in patients of lower socio-economic status than in higher, 63.2% and 37.8% respectively.

Table 1: Shows number and percentage of anemia (gm/dl) among males and

Gender	Total (%)	Females		
		Mild (%)	Moderate (%)	Severe (%)
Female	70.1	48.7	19.9	1.5
Male	53.2	34.3	17.7	1.2

Types of Anemia's

Iron deficiency anemia was considered present if the patient had low serum iron (lower than 50 ug/dl in women and 60 ug/dl in men), low ferritin (lower than 15 ng/ml), low transferrin saturation rate (lower than 16%) or increased total iron binding capacity (higher than 450 ug/dl). Anemia of chronic disease was defined as low circulating iron in the presence of increased iron stores (normal or increased ferritin higher than 100 ng/ml, transferrin saturation higher than 25% and lower than 50%) and decreased total iron binding capacity (lower than 250 ug/dl). Thalassemia trait was considered when the following conditions were present: low or very low mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), increased red blood cell (RBC) count, normal or increased circulating iron in the presence of normal or increased iron stores⁽⁶⁾. Anemia associated with folate or vitamin B12 deficiency was defined as concentrations of folate lower than 3.0 ng/ml or vitamin B12 lower than 200 pg/ml and MCV higher than 95 fl. Anemias that could not be classified into any of the previous categories were considered to be of unexplained origin. The hematologic characteristics

consistent with the possible presence of myelodysplastic syndromes were defined as macrocytosis in the absence of folate or B 12 deficiency, and leukopenia.

The incidence of neutrophilic leukocytosis with toxic granules was observed in 20.4% of cases. It was more commonly seen in females than males being 52.5% and 47.5% respectively. The relationship between toxic granules in mature neutrophils in infectious disease and inflammation has been described. The role of leukocytosis and neutrophilia in predicting bloodstream infection is also of questionable utility, as their etiologies include infection, stress, medication, trauma, and abnormal bone marrow production. These neutrophils show toxic granules with shift to left.

The incidence of thrombocytopenia in our study was 11.5%. Out of this, severe thrombocytopenia was seen in 6.3% of patients. Major causes of thrombocytopenia were severe infections, drug induced, gestational thrombocytopenia, sepsis and other hematological malignancies.

Also, 757 patients had pancytopenia on their peripheral blood smear that made a frequency of 7.57% out of the 10000 total admissions during the study period. Out of 757 cases, there were 450 (58.6%) males and 314 (41.4%) females patients with male to female ratio 1.5:1. Age range was 10–70 years, with a mean of 36.5 years, 16% being children the highest number of cases were found in the age group of 30 to 49 years (41.6%), 16.6% were in age group of <18 years and 6.2% were in the age group of >60 years. Megaloblastic anemia and infections such as enteric fever malaria, kala-azar and bacterial infections can be common causes of pancytopenia in developing countries [7]. Anisocytosis (75.1%) was the most important peripheral blood finding in pancytopenic patients followed by microcytosis(53.08%), dimorphism (46%), presence of normoblasts (16.8%), low leucocyte count and thrombocytopenia. Megaloblastic anemia was the commonest cause that was observed in 44.9% cases followed by infections like enteric fever causing pancytopenia being 25.4% , drug induced pancytopenia 13.4% . Other causes of pancytopenia include aplastic anemia ,erythroid hyperplasia, subleukaemic leukaemia etc.

Table 2: Showing types of anemia and their percentage distribution

Anemia	Total no of patients	total %	%
Thalassemia trait	321	9.1	
Thalassemia			5.9
Thalassemia+low vitamin b12/folate			3.2
Vitamin b12 or folate deficiency	356	10.1	
Low vitamin b12 and MCV higher than 95 fl			3.9
Low folate and MCV higher than 95 fl			4.2
Low vitamin b12 and folate and MCV higher than 95 fl			2.0
Iron deficiency anemia (IDA)	757	16	
IDA			9.5
IDA+ low vitamin b12 /folate			6.5
Anemia of chronic disease (ACD)	614	17.4	
ACD			8.1
ACD + low vitamin b12/folate			3.7
ACD + IDA			2.4
ACD + IDA +low vitamin b12/folate			1.4
Hematological reasons			1.8
Hemolytic anemia	530	15	
Hemolytic anemia			9.7
Hemolytic anemia + thalassemia			0.6
Hemolytic anemia + IDA and/or low vitamin b12 /folate			4.7
Unexplained anemia	931	26.4	
Unexplained			18.3
Possible myelodysplastic syndromes			6.1
Others types of anemia	21	0.6	
	Total 3530		

Leukaemia / myeloproliferative disorders were seen in 6.3% of the total study group. 32.8% of patients were having AML, 40% patients with CML, 15.2% patients with ALL and 12% with CLL. There were 70.4% male patients with chronic leukemia and 60.6% male patients with acute leukemia. Acute and chronic

leukemia had 29.6% and 39.4% female patients respectively. Among four major types of leukemia, ALL was observed in 60% male patients and in 40% female patients, AML in 63% male and 37% female patients, CML in 70% male and 30% female patients and CLL in 78% male patients and in 22% female patients. Acute lymphocytic leukemia (ALL) was more commonly noted in children (<15 years) whereas AML was more commonly noted in adults (>15 years). Both CML and CLL were observed only in adults (Table-2)

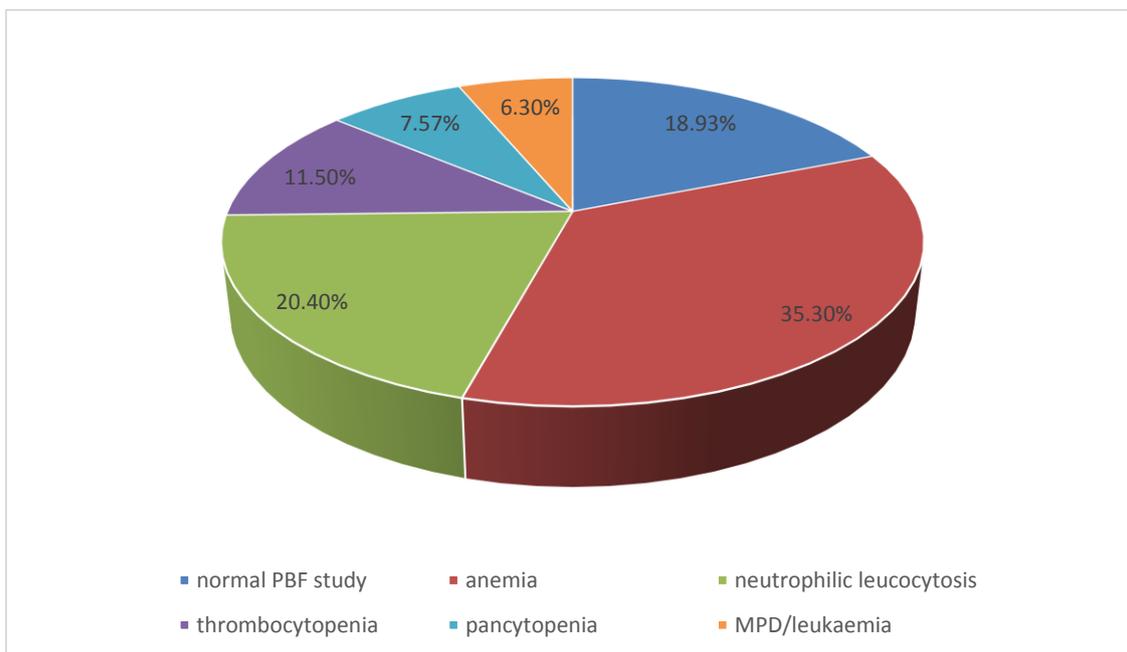


Figure 1: Pie Chart showing overall percentage of various type of findings in Peripheral Blood Film.

DISCUSSION

The present population-based study was specifically aimed at thoroughly investigating the epidemiology of anemia in an unselected population. Blood samples were prospectively collected for the purpose of the study and rates of inclusion were high. Findings from this large survey indicate that more than one out of ten persons were anemic and that most of the cases are of mild grade.

Total prevalence of anemia in our study was (35.3%) which is higher than the study done by the Collerton J. et al. (29.8%) [8]. In a study done by Shah BK et al in adolescent girls of Nepal, the prevalence of anemia (68.8%) was found to be lower than the Indian females in the present study (70.1%)[9].

In our study, the prevalence of anemia among males (20-50 years) was 53.2%, which was more as compared to NFHS 3 data 24% [10]. In a study conducted by Malhotra P. in 2004 and show the prevalence of anemia in the 16-70 years age group among males was 44.3%[11]. The present study highlights a higher prevalence of anemia in males and females as compared to previous data (Table-1).

In this study, neutrophilic leukocytosis with toxic granules was observed in 20.4% of cases .Kugeland Rosenthal described in 1932 the presence of large, dark, irregular,basophilic granules in the cytoplasm of neutrophilic granulocytes in patients with severe infection in comparison to the typical fine granular staining pattern. These were named as “toxic granules” (TG) and are helpful in predicting acute infection. In Liu et al. described the usefulness of these morphological changes, that the presence of TG was more predictive of bacteraemia than the total leukocyte count [12]. In our study, the incidence of thrombocytopenia was 11.5% which was slightly higher than reported by Dwivedi et al in 2012 observed the prevalence of thrombocytopenia as 8.5%[13].

The incidence of pancytopenia reported in our study was 7.57%. This incidence is quite variable as compared with other studies. According to a study conducted in Peshawar in the year 2000, it was 0.8%, while

Kanchanalak et al. and Adil et al [14] reported 1.2% and 12.6% respectively. Megaloblastic anemia was considered the most common cause of pancytopenia (44.9%) in our study. Similar results have been found by other local studies [15]. The high prevalence of nutritional anemia in India has been cited for the increased frequency of Megaloblastic anemia. In the present study, generalized weakness (74%) was the commonest symptom followed by fever and bleeding manifestations

Total incidence of leukaemia was 6.3%. Acute leukaemia (51%) was more prevalent as compared to chronic leukaemia (49%) and similar finding have been reported in previous studies. In our study commonest type of leukaemia was CML followed by AML, ALL, CLL. Previous studies have reported more cases of myeloid leukaemia [16]. A higher incidence of CML has also been reported by two earlier studies from India. Among lymphoid leukaemias, acute (17.2%) was common than chronic lymphoid leukaemia (10%) in our study with male prevalence. This has been reported by earlier studies [17]. The leukaemic blast in peripheral blood film was highest in AML with median blast cell count 45% followed by CML 42%, ALL 38%, CLL 35%. It was comparable with earlier studies.

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