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ETIOLOGICAL PROFILE OF MACROCYTIC ANEMIA IN PATIENTS ADMITTED IN PSG HOSPITALS

By

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Dissertation submitted to the

Tamil Nadu Dr. M.G.R Medical university, Chennai

In partial fulfilment of the requirements for the degree of

Doctor of Medicine in General Medicine

Under the guidance of

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CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled, **"ETIOLOGICAL PROFILE OF MACROCYTIC ANEMIA IN PATIENTS ADMITTED IN PSG HOSPITALS"** is the bonafide original work of **Dr. YOGANATHAN. C** in partial fulfilment of the requirements for the degree of Doctor of Medicine in General Medicine

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I hereby declare that this dissertation entitled **"ETIOLOGICAL PROFILE OF MACROCYTIC ANEMIA IN PATIENTS ADMITTED IN PSG HOSPITALS"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. Sujithkumar. S M.D,** Professor of Medicine, P.S.G IMSR, Coimbatore.

This dissertation is submitted to the Tamil Nadu Dr. M.G.R Medical University in fulfilment of the University regulations for the award of MD degree in General Medicine. This dissertation has not been submitted for award of any other degree or diploma.

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June 18, 2013

To
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The Institutional Human Ethics Committee, PSG IMS & R, Coimbatore -4, has reviewed your proposal on 28th May, 2013 in its expedited review meeting held at College Council Room, PSG IMS&R, between 2.00 pm and 4.00 pm, and discussed your application to conduct the study entitled:

"Etiological profile of macrocytic anemia in patient admitted in PSG Hospitals"

The following documents were received for review:

1. Duly filled application form
2. Proposal
3. Informed Consent form
4. Assent form
5. Parental Consent form
6. Data Collection Tool
7. Budget
8. CV

After due consideration, the Committee has decided to approve the above study.

The members who attended the meeting, at which your proposal was discussed, are listed below:

Name	Qualification	Responsibility in IHEC	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
Dr P Sathyan	DO, DNB	Clinician, Chairperson	Male	No	Yes
Dr S Bhuvaneshwari	M.D	Clinical Pharmacologist Member - Secretary	Female	Yes	Yes
Dr Sudha Ramalingam	M.D	Epidemiologist Alt. Member - Secretary	Female	Yes	Yes
Dr D Vijaya	Ph D	Member – Basic Scientist	Female	Yes	Yes

The approval is valid for one year.

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We request you to intimate the date of initiation of the study to IHEC, PSG IMS&R and also, after completion of the project, please submit completion report to IHEC.

This Ethics Committee is organized and operates according to Good Clinical Practice and Schedule Y requirements.

Non-adherence to the Standard Operating Procedures (SOP) of the Institutional Human Ethics Committee (IHEC) and national and international ethical guidelines shall result in withdrawal of approval (suspension or termination of the study). SOP will be revised from time to time and revisions are applicable prospectively to ongoing studies approved prior to such revisions.

Yours truly,

18.6.13

Dr S Bhuvaneshwari
Member - Secretary
Institutional Human Ethics Committee

SECRETARY
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INSTITUTIONAL HUMAN ETHICS COMMITTEE

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INTRODUCTION

Macrocytosis is common in various clinical settings and it is found in approximately 1.7–3.6% of people admitted for care for any cause[1,2,3]. Macrocytosis would be seen even in the absence of anemia. Heterogeneous group of disorders acting via various known and unkwown processes can lead to macrocytic anemia. Macrocytic anemia is generally classified as megaloblastic or non-megaloblastic anemia. Disorders that affect the synthesis of DNA in the precursors of erythrocytes leads to megaloblastic anemia and other disorders through various processes causes non-megaloblastic anemia.

Often we see macrocytosis preceding anemia[4,5,6], which is usually not investigated, particularly when anemia is very mild. Vitamin B12 deficiency may perhaps produce only low grade macrocytic anemia which when persistant for a prolonged duration there is a rapid detoriation, which has been demonstrated in various case studies. Patient might present with similar symptoms irrespective of the cause for anemia. An increased value of MCV, among various other findings at regular laboratory investigations, might be the early feature of various disease states like low vitamin B12 or low folate levels, pre leukemia, drug induced or alcoholism[7]. Macrocytic anema would be wrongly diagnosed as iron deficiency anemia in many of the situations because of similar presentation of variety of anemias. When there is no response to iron supplementation after a latent period then only the diagnosis of megaloblastic anemia is offered. Suspicion at high level, properly eliciting the history and thorough examination of the patient will lead us in diagnosing macrocytic anemia. To search for and identification of distinct clinical features may help to diagnose megaloblastic anemia and also may help in the early identification of low levels of B12 or folic acid.

AIMS AND OBJECTIVES

- To identify the etiology of macrocytic anemia in patients presenting to tertiary care hospital in South India (PSG Hospitals).
- To evaluate the causes for megaloblastic anemia.
- To evaluate utilization of bone marrow examination and upper GI endoscopy in diagnosis of megaloblastic anemia.

REVIEW OF LITERATURE

Anemia in humans have been classified on determining the mean corpuscular volume (MCV) of the red blood cells[8]. The size of the red blood cells is indicated by Mean corpuscular volume. Upon determining the MCV various pathologic processes might me thought and few others could be excluded. Electronic cell counters which determines the various parameters of the cells in the blood has made the MCV as a useful and integral part of the red cell profile[1,2]. MCV is the value of average volume of the red cells calculated by the coulter counter. It is not only used to classify anemia but also to identify the reason for low hemoglobin. Normal range of MCV is between 78 fl and 94 fl. If a patient has low hemoglobin and MCV of lower than 78 fl then he is diagnosed to have microcytic and if it is higher than 95 fl he is diagnosed to have macrocytic[3] anemia. Since lack of either folic acid or vitamin B12 disrupts the maturation process and causes megaloblastic changes in the precursor cells, deficiency of either of the vitamins produces same symptoms. The commonest cause of cobalamin deficiency is malabsorption. Inadequate intake through dietary source would be the other only cause. Vitamin B12 deficiency may also result if there is surgical removal of the stomach leading to non-availability of intrinsic factor or ileum leading to non-availability of absorptive surface[7,9,10]. Intestinal stagnant loop syndrome, diverticulosis of jejunum, ileocolic fistula and intestinal strictures may lead to cobalamin mal-absorption thereby causing deficiency. Infestation of the intestine with worms may lead to cobalamn deficiency particularly fish tape worm. Other common mal-absorptive cause is Tropical sprue seen in tropical region. Pure vegetarians are prone to Vitamin B12 deficiency than non-vegetarians. Children born to mothers with B12 deficiency are more prone and would develop B12 deficiency within about three to six months because of lower storage

levels. Congenital deficiency of intrinsic factor causes Pernicious anaemia a peculiar type of megaloblastic anaemia[9].

Folic acid deficiency is the most common vitamin deficiency disease which leads to megaloblastic anemia. Folate deficiency occurs commonly in the extremes of age i.e. in infancy and old age. Poor dietary intake occurs in patients in poverty, associated with scurvy and kwashiorkor. Mal-absorptive syndromes like Tropical sprue, gluten induced enteropathy may cause folate deficiency. Because of increased demand, associated with pregnancy, growing children and in people with haemolytic anaemias deficiency of folic acid occurs.

The presenting features of patients with megaloblastic anemia like anorexia, irritability and easy fatiguability are due to anemia. Other symptoms like loss of weight, diarrhea or constipation could be present. Distinct clinical findings associated with megaloblastic anemia are reversible hyperpigmentation, angular cheilosis and glossitis[11]. There may be enlargement of liver and spleen and sore tongue. Severely anemic patient may present with mild fever and jaundice. Associated low platelet count and low leucocyte count leads to bleeding tendencies and infections as the presenting symptoms.

Deficiency of vitamin B12 causes symmetrical peripheral polyneuropathy, loss of myelin in the spinal cord particularly involving the posterior column and pyramidal tracts. Neurological features seen in Vitamin B12 deficiency are parasthesia in fingers and feet, memory loss, poor gait, loss of position sense, psychiatric disturbances, blindness and optic atrophy. Folic acid deficiency do not produce neurological symptoms[12].

Peripheral blood smear in early stage before producing anemia may show only few macrocytes and hypersegmented neutrophils. Later stages show oval macrocytes, anisocytosis and poikilocytosis. Reduction in total leucocyte and platelet counts are noted in parallel to the degree of anemia.

Bone marrow is hypercellular. The erythroblast nucleus maintains a primitive feature. The cell size would be of greater than normoblasts and there might be more number of cells with eccentrically placed nuclei with lobulations or nuclear fragments might be seen. The other characteristic features are enlarged and differently shaped metamyelocytes and large sized hyperploid megakaryocytes.

Macrocytosis with increased MCV is seen in chronic immune mediated haemolytic anaemia[13]. It is due to an increase in reticulocyte count. Bone marrow undergoes a megaloblastic change due to an increased demand of folic acid for hyperplastic erythropoeisis. Overall, a picture of ineffective erythropoeisis is seen which leads to destruction of cells causing pancytopenia. Investigations reveal elevated serum unconjugated bilirubin and elevated serum LDH level. Megaloblastic anemia is associated with more anisocytosisis than that of non-megaloblastic anaemia.

There have been many drugs that have been implicated in causing macrocytosis and they are cyclophosphamide, hydroxyurea, methotrexate, pyrimethamine,trimethaprim, valacyclavir etc.

In evaluation of macrocytosis microscopic examination by peripheral smear is a much more sensitive when compared to automated RBC indices. Automated RBC indices underestimate about 30 % of cases of macrocytosis when compared with peripheral smear finding.

Hyperglycaemia, cold agglutinins and marked leucocytosis causes falsely elevated MCV[14,13,15]

Macrocytosis without anaemia can also be a normal variant and is noted to occur in families with a genetic predisposition. The common pathologic causes for macrocytosis are

1. Drugs
2. Alcoholism
3. Reticulocytosis
4. Hypothyroidism
5. Liver diseases
6. Low Vitamin B12
7. Multiple myeloma
8. Folate deficiency
9. Myelodysplastic syndromes
10. Acute leukemia
11. Aplastic anemia

Hypersegmentation of neutrophils signifies megaloblastic “arrest” and thus impaired DNA synthesis. The following are the feature of hypersegmentation:

- Any neutrophil with 6 or more lobes,
- 5% of neutrophils with 5 lobes,
- majority have more than 4 lobes.

A lobe is considered distinct if it is separate from the nucleus or connected by a fine chromatin thread. It has been suggested that hypersegmentation can be most reliably detected by use of the segmentation index (% of neutrophils with five lobes or more, relative to the number of four-lobed neutrophils).

Other diseases associated with Hypersegmented neutrophils are as follows:

1. Iron deficiency anaemia[16,17]

2. Uraemia[18]

3. Hyperthermia[19]

4. Myelodysplastic syndromes[20]

5. Langerhans cell histiocytosis[21]

6. Post irradiation[22]

7. Drugs such as chemotherapeutic agents, steroids[23], granulocyte colony stimulating factor.

Different studies have reported variable incidence of hypersegmented neutrophils in megaloblastic anaemia. The incidence of hypersegmented neutrophils in a study in Chinese population by Chan et al shows the incidence to be about 68%[24].

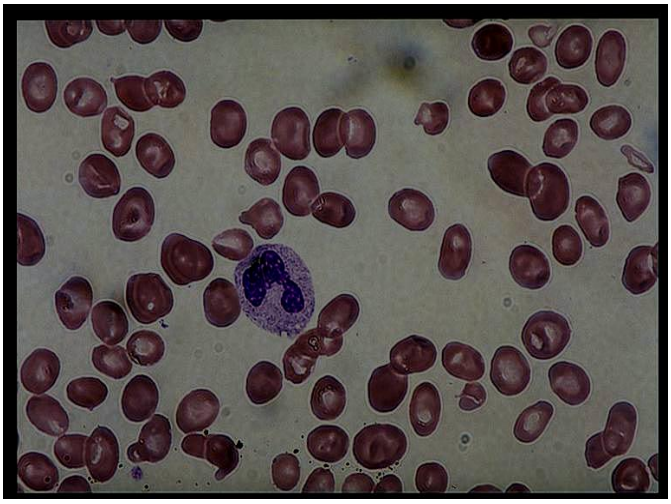


Figure1: Peripheral smear picture in a patient with megaloblastic anaemia showing a hypersegmented neutrophil and macrocytosis

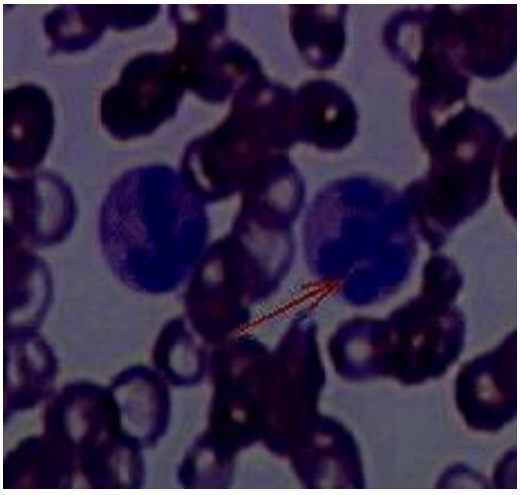


Figure 2 Peripheral smear picture with hypersegmented neutrophils

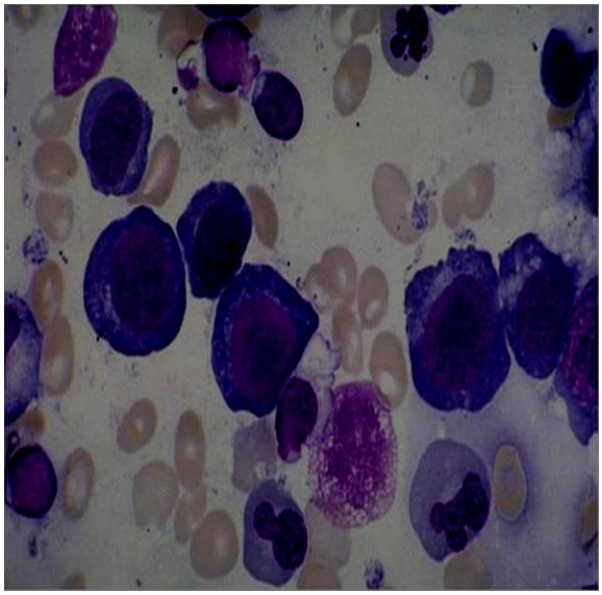


Figure 3: Bone marrow picture in a patient with megaloblastic anaemia

DIAGNOSIS OF MEGALOBLASTIC ANAEMIA

Determining the serum level of vitamin B12 is the preliminary step in evaluation of megaloblastic anaemia. Quantaphase Radio II assay which is an older and an non-automated protein binding method was subjected to various studies, has sensitivity of 95-97%.[25] Nowadays it can be done in a fully automated method. This method is expected to have sensitivity atleast as that of non-automated method. Excluding wholly vitamin B 12 deficiency inspite of normal serum levels of B12 not entertained. The following are the conditions in which there would be more than expected level or normal vitamin B12 levels even in the face of deficiency:

1. Renal failure
2. Liver disease
3. Chronic myeloid leukaemia (CML) and also other myeloproliferative disorders
4. Nitrous oxide inhalation
5. Congenital transcobalamin II deficiency.
6. Metabolism errors of intracellular B12
7. Parenteral therapy with Vitamin B12 supplements

In elderly population Methyl malonic acid along with total plasma homocysteine, considered as B12 related metabolites are found to be elevated in decent proportion of patients with low normal or high normal B12 levels in the serum. One of the study has stated that 35% of their study population with low-normal B12 levels, i.e. the levels between 140–258 ng/l, and 24% of study population with high normal levels, i.e. the level >258 ng/l, had increased levels of serum methyl malonic acid and plasma total homocysteine. But the high levels of MMA was seen only in 12 and 11% of the population, respectively[26]. In view of this few researchers have advised complete evaluation of status of serum B12 in the patients who has B12 levels

are higher than the lower 95% upper limit for vitamin B12. If it done so i.e practicing this methodology might cause unnecessary evaluation and testing of many individuals among whom there might not be deficiency of B12 vitamin and certain fraction of persons in whom there might be subclinical deficiency[27]. Some of the conditions are often found to be associated with reduced vitamin B12 assay levels in spite stores of the vitamin is well with in normal

1. Pregnancy
2. HIV infection
3. Folate deficiency
4. Patients on anticonvulsant drugs
5. Myeloma
6. Transcobalamin I deficiency
7. Unexplained

Mild and occasionally severe TC I deficiency[28] may cause low serum B12 levels in about fifteen percent of general population of patients and low levels of B12 without malabsorption or any abnormality seen in the B12 metabolite. Determination of the presence of Transcobalamin I deficiency is made by Radio immune assay. Methylmalonic acid and plasma total homocystreine levels are increased in the face of vitamin B12 deficiency. The problem with Methylmalonic acid assay is that it is of high cost, accessibility and a duration taken to report is significantly longer. Provided that renal function has been ruled out, estimation of serum MMA levels would be of highly indicative of low B12 levels. 2% of

cases with folate deficiency and 98% with vitamin B12 deficiency have elevated Methylmalonic acid levels[25]. Homocysteine assays are less expensive but the availability is wider when compared to serum Methylmalonic acid assays. The sensitivity of homocysteine assay is 96% 37 and 91% in cases of B12 and folate deficiency respectively. Estimating serum homocystein levels might be helpful in the following patient population i.e. Kidney injury with reduced GFR, over consumption of alcohol, low level vitamin B6, hypothyroidism, patients taking drugs like Isoniazid and defects in homocysteine metabolism. Supplementation of vitamin B12 to the deficient patient may normalize their methylmalonic acid and homocysteine levels but it would not occur when they are supplemented with folate[29].

Holotranscobalamin II (holo-TC II)

Transcobalmin II is a protein that transports B12 and supplies it to the cells for metabolism. This happens in about 6–20% of the total serum B12. Transcobalamin I (haptocorrin) binds the rest of all the B12 whose function is not evaluated in full. Above finding has enabled us to know that estimating the level of TC II-bound B12 (holo-TC II or holo-TC) would provide us the exact amount of vitamin B12 available for cells for metabolism. In earlier days were older methods were followed for estimating holo-TC II it was noted that the levels were interfered with other conditions than that of B12 status affecting in specificity. It has been found by various researchers the new radioimmunoassays is more sensitive than estimating B12 levels in establishing the diagnosis in individuals with more than normal levels of MMA and tHCYS levels[30]. Before using holo-TC II further research work is needed as there are conflicting data on specificity of using and further studies are required before the value of holo-TC II in determining B12 status is established.[31,32,33]. Eighty nine percent was specificity of this assay as identified by one of the study. Chen et al [34] after his research work recently

stated that deficiency of B12 was much more important than impaired B12 absorption in measuring the level of holo-TC II. Significant amount of overlap was noticed within serum holo-TC II levels among the patients who were treated for pernicious anaemia and control patients who does have the disease. This was in contradiction to older suggestions, finally concluding that estimating and identifying holo-TC II levels may not be used as a surrogate for the Schilling test.

DEOXYURIDINE SUPPRESSION TEST (DUST)

The efficiency of the hematopoetic cells involved in the metabolism of converting deoxyuridylate to thymidylate by the process of methylation is identified by the above test. It have been found that it is been abnormal in both B12 and folate deficiency [35,36,37,38].Worldwide, it is found that this test is being performed only in few of the laboratories. The DUST is more sensitive in detecting B12 deficiency than MMA or tHCYS levels[38]. It has the main draw back since it is being performed by using bone marrow cells which might involve an invasive procedure.

BIOCHEMICAL TESTS FOR ASSESSING FOLATE STATUS IN SERUM AND RED CELL

In human body the storage status of folate is evaluated by measuring the level of folate in the serum or else in the red blood cells. Majority of the proportion of folate in the serum would be in the form of 5-methyltetrahydrofolate monoglutamate. Likewise in the red blood cells folate would be in the form of polyglutamates. Since the life time of red blood cells are about three months measurement of red cell folate would give us an estimate of the vitamin over a period of three months. In general since the folate level in the serum mostly reflects the current or short term intake of the vitamin its measurement is mostly useful identifying recent

folate deficiency[39]. Various other research works had showed statistical correlation among the values of folate in the serum and red blood cell which was done by using microbiological and fully-automated methods[40,41]. Based on the above reports few of them stated that folate level in both serum and red blood cell might offer same amount of clinical information. In the studies it was found that there was a weak correlation. By analyzing the statistics in the studies conducted by Jaffe & Schilling[41] and Phekoo et al[40] it is observed that only 30% and 24% respectively in the above studies , the variation of the serum folate can be accounted by variations of red cell folate.

In estimating the folate levels the precision of folate assays, predominantly of fully-automated red cell folate assays is debatable. It have been stated that the results obtained depend on what method was used for estimating the levels i.e by microbiological method or radioassay or fully-automated or GC-MS[42,43]. This type of variation in the results when different methods are used is because the haemolysate preparation differs and there is difference in deconjugation of folate poly glutamates to mono glutamates. Since the red cell derived mono glutamates contains combination of various species it would be tough to make assay design.

It has been found that nearly sixty percent of the patients with low B12 have their red cell folate to be low. This makes the red cell folate assay to be low specific in diagnosing folate deficiency. But in those patients twenty percent had increased serum folate levels and ten percent had low serum folate levels. When patient has normal B12 and low folate level, in clinical practice then only the patient is said to have folate deficiency. When both folate and B12 are found to be low firstly the possibility of B12 deficiency is considered instead of

folate taking into the consideration of the clinical scenario. It would be advisable to do a MMA assay or Schilling test can be done.

We cannot expect metabolic abnormality of folate or B12 in all patients presenting with reduced red cell folate levels. Only 8 out of 45 patients with macrocytic anemia was found to have low red cell folate in one of the study. Radio assay was used in this study. Those patients had normal DUST results[44].

Plasma total homocysteine (tHCYS) and serum/plasma methylmalonic acid (MMA)

As we are aware by previous discussion tHCYS is elevated in both folate and B12 deficiency. Hyper homocysteinemia is recovered when such patients are supplemented with folate than that of B12[29]. The levels of MMA is not affected by low folate levels.

TREATMENT

Cobalamine deficiency

Apart from specific therapy related to the underlying disorder, the mainstay of treatment for cobalamine deficiency is replacement therapy. Parental treatment begins with 1000 micro g cobalamine daily for 1 week followed by weekly for eight weeks and then monthly once for the rest of patient's life.

Folate deficiency

As for cobalamine deficiency, folate deficiency is treated by replacement therapy. The usual dose of folate is 1 mg/d by mouth, but higher doses (upto 5 mg/d) may be required for folate deficiency due to malabsorption.

OTHER CAUSES OF MACROCYTOSIS

HYPOTHYROIDISM

Prevalence of hypothyroidism have been reported to be 2-5% worldwide even though the incidence varies among the different geographic area**.** Conversely, subclinical hypo thyroidism has the prevalence roughly between 4 to 8.5%. It is also noted that prevalence of sub-clinical hypo-thyroidism would rise upto twenty percent in women in the age group of sixty or more older[45].

Metabolic deceleration occurs in the state of hypothyroidism. All the organs in the human body is affected .The manifestation of thyroid deficiency, symptoms and findings would be different characteristics which depends on the age of onset of the disease and also the deficiency or inefficiency of the thyroid hormones.

Among many manifestations of this disease state anemia would be most common and important presentation since it affects the hemotopoietic system. Mediocre anemia is commonly seen in hypothyroidism. About 20 to 60% patients are said to have anemia in hypothyroid state.

Various types of anemia like normochromic normocytic, hypochromic microcytic, and macrocytic would be a presenting feature in hypothyroid. It is also stated that the level of haemoglobin drop depends upon the severity of thyroid deficiency. Findings of hypocellular features in the bone marrow indicates that thyroid hormone also plays an important role in hamatopoiesis.

In a normal clinical setting normochromic normocytic anemia is most commonly seen. The most common cause is the bone marrow suppression due to low level of thyroid hormone and also insufficiency of erythropoietin secretion or production because of decreased oxygen demand. Life cycle of the red blood cells is found to be normal in hypothyroid state and also there would be hypoproliferative erythropoiesis. Thyroid hormones are necessary in production and it also increase 2-3 DPG (diphosphoglycerate) levels supporting in the delivery and diffusion of oxygen inside the cells [46,47,48].

Patient would present with features of various other autoimmune disorders if the patient has autoimmune thyroid disorders. Pernicious anemia an auto immune disorder might complement thyroid deficient state as a component of polyglandular auto immune syndrome. The theory proposed as a cause for macrocytic anemia in thyroid deficient state is that intrinsic factor deficiency and absence of hydrochloric acid in gastric secretion leads to poor or nil absorption of B12 vitamin. Another study states 55% of the hypothyroid patients have associated Macrocytosis[49]. Iron deficiency anemia is linked with menorrhagia happening as a result of various hormonal disproportions and also malabsorption which is seen in hypothyroidism[50]. In hypothyroidism[51] reduced absorption of folate is also noted to be the triggering factor for anemia with macrocytosis.

MEGALOBLASTS IN ALCOHOLIC

In chronic alcohol consuming patients occurrence of megaloblasts in their marrow is seen. The above feature is noted in approximately one third of the patients consuming alcohol who has presented with features of anemia[52]. It is found that persons who consume alcohol chronically will have low folate in their red blood cells. The important reason for this

deficient state is consumption of food very much low in folate levels. Because of their poor dietary pattern this is most commonly seen in persons consuming alcohol. Added to this poor eating pattern consumption of alcohol by itself leads to foalte deficient state affecting the absorptive ability of intestines of folate present in the diet.

The aberrations in synthesis of red blood cells evidenced because of alcohol consumption is not exclusively found in marrow but also seen in peripheral blood by the existence of abnormal red blood cells.

Obviously large sized red blood cells seen in the peripheral blood – a feature named as macrocytosis along with morphologically unusual red blood cells that are undergoing early or hastened demolition a phenomenon called as haemolysis occurs due to their abnormality in the structure. Due to the above fact patients with chronic alcohol consumption most often have the diagnosis of macrocytic anemia.

It is been noted that comparing the mean corpuscular volume of the red blood cells using automated machine for counting the cells between the patients who consumes alcohol and who does not, the patients who consumes alcohol had their MCV to be higher. Nonetheless, when the MCV is high macrocytosis should not be made as a diagnosis routinely. Occurrence of bigger red blood cells in the peripheral smear not only meant because of alcohol but there are many diverse diseases. It is also very essential to examine the red cells using a microscope in order to establish a diagnosis of macrocytosis, and also to recognise the various abnormal structures distinctive for various other specific diseases. Hence the bloated RBC's in persons having macrocytosis usually are homogenously round, where in oval

shaped red blood cell are predominantly seen in anemia with megaloblastosis. Apart from that, alcoholism leading to macrocytes in the blood could be diagnosed only after exploring all the prospective reasons for enlarged red blood cells along with the history of consuming alcohol[53].

Production of red blood cell is found to be reduced by alcohol and also due to cirrhosis caused by alcohol. Hypersplenism, a situation characterized with increased size of the spleen and reduced number of red blood cells or WBC or platelets or all can cause untimely demolition of red blood cells. Principle site of loss of blood is found to be through intestines and is amplified in those persons with lower platelet counts. Postulated causes of anemia in alcoholism are[54]

- Hypersplenism
- Losing Blood
- Liver disease
- Low folate levels
- Lower red blood cell synthesis

People who drink too much amount of alcohol can have enlarged red blood cells i.e macrocytosis inspite of other contributing reasons for increased red cell size found in consuming alcohol chronically like cirrhosis or other liver disorders associated with alcohol consumption or deficient folate. Its true that, excessive consumption of alcohol is the ailment frequently connected with enlarged red blood cells. Alcoholism was noted to be associated with enlarged red cells in up to eighty percentage of men and forty six percentage of

women in one study. The exact process leading to enlarged red cells is not known till now. Nevertheless it is noted there is direct interference of alcohol in growth of red blood cells, since the enlarged red cells vanish between two to four months of self-restraint.

Lysis of red cells might be a fundamental factor leading to anemia, and numerous types of haemolysis may be triggered by consumption of huge amount of alcohol for longer period. Among numerous type of red cell destruction two of them are associated characteristically with the existence of abnormally shaped red cells called as stomatocytes and spur cells[55], although lysis of red cells occurs due to low phosphate levels in the serum because of chronic alcohol consumption.

Diagnosis of red cell destruction in patients with alcoholism is demanding, since these persons commonly show other associated illnesses, like as alcohol withdrawal, low serum folate levels, blood loss and large sized spleen. When cells are seen under a microscope stomatocytes are seen as mouth or stoma shaped, this is because of fault in the red cell membrane. Destruction of the this abnormal stomatocytes occurs in the spleen as they get caught in the smaller capillaries within the spleen following which cells are destroyed leading to an abbreviated life span for red cells. Even in normal persons less than five percentage is occupied by stomatocytes in the total red cell count. However the percentages are found to be considerably greater in alcohol consuming persons. Indeed, greater than twenty five percentage of persons consuming alcoholic chronically will display an amplified percentage of stoma shaped red cells called as stomatocytosis[56].

The precise process that leads to the production of stomatocytes in alcoholics is quite uncertain. Liver disorders associated with alcohol consumption could be one of the causes in the production of stomatocyte.

SPUR-CELL HEMOLYSIS[55]

Spur cells are distorted RBC's that are characterized by spike like protrusions of their cell membrane. These spurs are produced by the integration of superfluous amounts of cholesterol into the cell membrane, ensuring an increase of the cell's surface area without a concomitant increase in cell volume. Modestly elevated membrane cholesterol levels result in a flattened RBC shape, however larger increments of cholesterol cause the membrane to be thrown up into spikes. Spur cells may be prematurely eliminated in the spleen. Spur-cell hemolysis occurs in about 3 percent of alcoholics with advanced liver disease, causing anemia that progresses relentlessly and is eventually fatal. Clinicians have tried unsuccessfully to treat the disorder using various agents with cholesterol-lowering properties. Consequently, surgical removal of the spleen is the only treatment capable of slowing the hemolytic process. Most alcoholic patients with spur-cell hemolysis, nevertheless, are not tolerable candidates for major abdominal surgery, since their coexisting advanced liver disease increases their risk of bleeding. Moreover, the procedure may precipitate liver failure

HYPOPHOSPHATEMIA

Even though hypophosphatemia- induced hemolysis is uncommon, its most common cause is alcoholism, particularly in the course of the withdrawal phase. Phosphate is an indispensable element of adenosine triphosphate (ATP), a compound that affords energy for numerous cellular processes. Alcohol effects phosphate to be expelled with the urine. Intense hypophosphatemia may cause the phosphate and ATP levels in the RBCs to drop markedly.

This reduction of the store of ATP in the RBC's leads to amplified rigidity of the RBC membranes, ultimately damaging the cells. These damaged cells are in advance, destroyed in the spleen, and the patient may progress acute hemolytic anemia.

PREGNANCY INDUCED MACROCYTOSIS

The association of anemia and pregnancy is well familiar. In this country the anemia is frequently due to iron deficiencies and is hypochromic. In the tropics a macrocytic, hyperchromic anemia of pregnancy has fascinated consideration and has been the theme of many current investigations. Its complement in the temperate climates, the macrocytic or so-called pernicious anemia of pregnancy, has been considered a comparatively infrequent dyscrasia. The macrocytic anemia of pregnancy was first recognized over a century ago when Channing described ten mortal cases. In 1919 Osler distinguished the so-called pernicious anemia of pregnancy from classic Addisonian anemia by indicating out that when recovery from the former disease took place it was everlasting and the woman might escape it in succeeding pregnancies.

Anemia associated with pregnancy could be a significant problem faced in public's health causing substantial influence in increasing the mortality and morbidity among the mothers and child perinataly. In India with in a period between 1992 and 1994[57] world health organisation described the link of anemia in about sixty four percentage death among the pregnant women. Pregnancy alone may by it self lead to anemia by producing a situation of hydremic plethora. It can be noted there might be an unbalanced rise in volume of the plasma as equated to the masses of the red blood cells in pregnant patients causing an visible drop of count in the red blood cells, hemoglobin and hematocrit value. The peripheral smear would show normo chromic and normo cytic anemia. This is pretended physiological anemia of

pregnancy. Other nutritional deficiencies like iron, folate, B12 vitamin and protein can aggravate and complicate this state of physiological condition. It is also noted anemia would also result from other associated factors hemolysis and bleeding disorders, also due to losing blood acutely or chronically. Predictably, anemia due to low serum iron is being equated with anemia of pregnant women and various literatures has recognised it. Recent studies[58,59] also found the same.

Anemia with macrocytosis contributes significantly to the anemia of different causes even though the occurrence and cause may not be consistent among given population. With growing occurrence of anemia in the community, it can be anticipated that the prevalence of macrocytic anemia among pregnant women would also be showing a growing trend. Amazingly, only low number of studies are existing in order to establish this fact, details on profile of the participant people and outcome of the pregnancy in those patients.

Studies conducted with in the general Indian public have shown upto sixty percent low B12 vitamin prevalance and 2.4% of anemia patients have peripheral smear study of macrocytosis[60,11], but surprisingly no researches have reported it at the time of pregnancy. This might have happened because higher occurrence of iron deficiency anemia has outshined it. Only study that could be seen in the literatures is a Venezuelavian study that deals with the issue of anemia with macrocytosis among pregnant women. This study disclosed that upto 36.32% study population had low folate levels and upto 61.34% had low B12 vitamin levels in pregnancy. In disparity, a study conducted in Americans the it was only between 3 to 4 percentage among the women with anemia with macrocytosis. Dietary pattern of the Americans was found to be the important reason for this lower occurrence of folate and

B12 vitamin deficiency which are the foremost cause for anemia with macrocytosis[61]. Their food has the richest sources of those vitamins.

DRUG

Our knowledge on various pathogenesis of blood disorders caused by ingestion of drugs has improved as years have been progressing, principally that involves RBCs. Anemia due to lysis of red blood cells is the utmost common finding. Lysis of red cells occurs because drug might damage the red cell which activate an immunopathognetic mechanism or by the interruption of enzymes involved in the metabolic process of the red blood cell. The immune source of drug causing lysis of red cells is considered significant as on the presentation of the patient and manifestation of lab results, various other diagnostic options and it is the foremost pathogenesis involving in lysis. Drug that causes lysis of red cells by inducing oxidative stress occurs both in normal persons and also in persons deficient of certain enzymes. Particularly glucose-6-phosphate dehydrogenase deficient persons have red cell lysis more commonly. Few of the drugs can induce red cell abnormality by affecting young red cells in the marrow. By an ill known process these drugs affects and stops the development of red cells. Other drugs might produce a particular effect on the metabolism of red cell pre cursors. Those are the drugs that comprises of a process that hamper the production of DNA and producing megaloblastosis during erythroid synthesis and those that interrupt function of the mitochondrium and the production of heme leading to sideroblastic erythropoiesis. The following are the drugs leading to sideroblastic anemia: anti tuberculous drugs, heavy metals like lead, ethyl-alcohol and chloramphenicol.

Many drugs used in therapeutics are implicated to cause adverse drug events by disturbing the haematological synthesis in animals, found in various studies. Adverse drug events is

been characterized as Type-A or Type-B reaction. Type-A adverse drug reactions have the characteristics of are dose dependency, which might be increased in certain group of the people. Type-B adverse drug reaction are characterised by idiosyncracy that they are not related to the drugs pharmacological effects. Drug adverse events caused by idiosyncrasy are more perplexing to explain because they are not able predict their effect and the process involved are many more. It is found that an exclusive genetic and or acquired proneness of the particular person is commonly intricated. Predisposition of the person genetically for a drug adverse event has mutations that change metabolic process involving the drug or persuade immune reactions against the particular drug or else on its metabolic products. Because of a change in the homeostasis that occurs because of the altered or abnormal function of the liver or the kidneys metabolic process of the drugs or their end product excretion are changed there might be acquired predisposition to adverse drug reaction.

Prognosis of the adverse drug reaction depends on the organ and process by which the drug affects. Nevertheless finding a specific site of the effect or the process by which it occurs is tough since many number of sites and many process are involved. Locations of ill effect comprise the various component of the blood and various component of the bone marrow which includes stem cells involved in hematopoiesis, their multiplying component cells and stromal tissue[62,63] present within the marrow. Red blood cells are mainly predisposed to injuries caused by oxidation stress[64]. Various components of the marrow and peripheral cells also look as if they are exceptionally predisposed to immune induced lysis. This predisposition can be linked to the truth we know the binding of the antibody occurs on the blood cell surface in normal subjects as a way of finding and eliminating the older age cells. Consequently adverse events having immunological mediation might be considered as abnormally skewed immune process. Marrow precursor cells and cells undergoing proliferation are briskly dividing cells and hence they are predisposed to chemo therapeutic

drugs. Capillaries and sinusoids present in the marrow cavity are susceptible to the damage Damage to the vessels could be appreciated in marrow cavity by visualising swelling of the interstitium, bleeding, cell death, fibrosis of the marrow and various inflammatory cells in the core biopsy specimen.

EVALUATING A PROBABLE ADES WITH EFFECT ON HEMATOLOGY

The temporal relationship of a drug intake with a hematological effects do not by itself offers confirmation of an adverse event. Other prospective factors that might lead to such a hematological situations might be excluded by appraisal of the past and present history, features noted during physical examination and clinicopathological results.

When neutrophil count is less than 1500 it is referred as neutropenia. Neutropenia is said to be an effect of adverse drug reaction when it has occurred with history of drug intake. Adverse drug event as a cause of neutropenia would not be drawn as conclusion when it has occurred within about four weeks of stopping the drug or has occurred more than four weeks after starting the drug if there was no leukocyte count done in the interval period. An elevation of the neutrophil count more than 1500 cells per micro-litres within four weeks after discontinuing drug intake is also in favour of adverse drug event.

When the platelet count drops below one lakh it is called as thrombocytopenia. Manifestation of thrombocytopenia within four weeks of starting a drug or remission within three weeks after discontinuing drug is indicative of ADE[65].

Depending upon the species and animal breeds the description of drug induced anemia differs. Moreover, the rapidity of developing anemia would depend on the process through which the drug causes anemia. Drugs that cause lysis of red cells intravascularly commonly leads to a speedy and increased severity in anemia with accompanying jaundice and

haemoglobin in the urine. Drug that causing lysis of red cells extravascularly is linked with a somewhat less speedy beginning of anemia and haemoglobin in urine and jaundice would be commonly lacking. Anemia would be of slow in progression when the drug leads to a hindrance to the erythropoietic process.

TROPICAL SPRUE

Tropical sprue is one of the more mysterious and rather poorly understood disease till now seen in tropical region. It is usually a clinical syndrome with an unknown etiology .Tropical sprue has more characteristically an acquired chronic diarrheal illness, associated with small bowel mucosal abnormalities which may result in malabsorption leading to nutritional deficiencies and loss of weight[66]. Many of these symptoms are vague which are seen in large number of other diseases. Diagnosis of tropical sprue is quite challenging as the endoscopic findings and histology of the specimen obtained from endoscopy is mostly non specific[67]. "Idiopathic Mailabsorption of the tropics" is the term used to describe the patients with chronic diarrhea by Dr.William Hillary in 1795[68]. It was broadly defined as malabsorption of two or more substances in people in the tropics when other causes have been excluded by Baker, in 1974[66,67].

Ghoshal, who has extensively studied on Tropical sprue over the past decade has often quoted Klepstein's definition, which focused on very specific criteria. Individuals must have the following features to meet the criteria: chronic small bowel diarrhea with malabsorption of two unrelated substances (i.e. carbohydrates and fatty acids), abnormal small intestinal histology, excluding the other known cause of malabsorption and consistent results to the treatment. Most important point in the definition is that the patient should be symptomatic[69]. It was redefined by Klepstein, which excludes the asymptomatic, healthy individuals who are

living in tropical region and also demonstrate villous abnormalities[66]. Patient presenting with these features are classified as having tropical enteropathy instead of TS. Patients with tropical enteropathy have features of subclinical malabsorption, they neither have diarrhea nor show clinical evidence of malnutrition[70],[71],[72].

It is known that the tropical sprue is prevalent in our country and even in Indonesia, Burma, Borneo, Singapore, Malaysia and Vietnam. But in China, Africa and Middle East it is less well known[73]. Tropical sprue has rarely been seen in the literature in United State. There are many factors to support that tropical sprue is due to infection and they are as follows[74,75]

1. Acute diarrhoeal disease, characteristically an infectious diarrhea often preceeds the disease.

2. Following the epidemics of diarrhea few patients were found to develop tropical sprue in epidemic.

3. Toxin producing coliform bacteria is found to be over grown. (Enterobacter, Escherischia coli and Klebseilla)

4. Usage of broad spectrum antibiotis cures the disease.

Bacterial overgrowth causes fermentation and release of toxigenic substances as a byproduct might cause small intestinal mucosal injury resulting in malabsorption.

1. Alcohol produced as the product of fermentation inhibit folate absorption leading to folate deficiency.

2. As the disease process involves the terminal ileum leads to malabsorption of Vitamin B12 and it becomes deficient.

3. Deficiency of disaccharide enzymes such as maltase, sucrose and lactase lead to Carbohydrate malabsorption

4. Malabsorption leads to Vitamin D deficiency which often causes hypophosphatemia and hypocalcemia.

5. Magnesium deficiency may occur.

6. Alteration in the villous architecture may cause fat malabsorption leading to steatorrhoea

Tropical sprue most commonly presents with

1. Diarrhoea

2. Easy fatiguability due to megaloblastic anaemia because folate and or vitamin B12 malabsorption.

The other clinical features may include chelitis, glossitis and pedal edema. Neurological manifestations of vitamin B12 deficiency in tropical sprue are rare. Procedure like upper GI endoscopy with duodenal biopsy is often used for diagnosis. Large intestine may also be involved in Tropical sprue. Scalloping and flattening of duodenal folds are the endoscopic findings that have been originally thought to occur in Celiac disease also occurs in Tropical Sprue[76, 77]. Eventhough spontaneous recovery from mild tropical sprue have been reported, most authorities recommend treatment with tetracycline (250 mg PO four times daily) plus folic acid (5 mg/day) for three to six months. Relapses or reinfection occur in up to 20 percent of patients living in the tropics when treated with this above regimen.

Most of the signs and symptoms of tropical sprue reverses with supplementation of oral folic acid[78]. Most of the patients treated with effective dose of folic acid (1 to 5 mg/day) within several weeks, show increased sense of well being, weight gain and improvement in anemia. This type of dramatic symptomatic response occurs more frequently to oral folate therapy in tropical sprue and so rarely (if ever) in other forms of small bowel disease with megaloblastic anemia that it is considered to be diagnostic of the illness. Morphologic abnormalities noted in the small intestines which suggest villous atropy could be the aetiology in tropical sprue is also corrected by the replacement of folic acid. This also suggests that villous atrophy is due to folate deficiency.

Small-Bowel Mucosal Histology in Certain Malabsorptive Disorders

Disorder	Histologic Characteristics
Normal	Fingerlike villi with a villous:crypt ratio of about 4:1; columnar epithelial cells with numerous regular microvilli (brush border); mild round cell infiltration in the lamina propria
Celiac disease (untreated)	Virtual absence of villi and elongated crypts; increased intraepithelial lymphocytes and round cells (especially plasma cells) in the lamina propria; cuboidal epithelial cells with scanty, irregular microvilli
Intestinal lymphangiectasia	Dilation and ectasia of the intramucosal lymphatics
Tropical sprue	Range from minimal changes in villous height and moderate epithelial cell damage to virtual absence of villi and elongated crypts with lymphocyte infiltration in the lamina propria
Whipple disease	Lamina propria densely infiltrated with periodic acid-Schiff–positive macrophages; villous structure possibly obliterated in severe lesions

Table 1: Small bowel mucosal histology in various malabsorptive disorders

Symptoms of Malabsorption

Symptom
Anemia (hypochromic, microcytic)
Anemia (macrocytic)
Bleeding, bruising, petechiae
Carpopedal spasm
Edema
Glossitis
Night blindness
Pain in limbs, bones, pathologic fractures
Peripheral neuropathy

Table 2: Symptoms of malabsorption

LACTATE DEHYDROGENASE[79,80]

Lactate de-hydrogenase iso-enzymes in human beings are chiefly utilised by cellular components in particular tissues. Human LDH Iso-enzymes, which is even called as lactic de hydrogenase, are accountable for transforming lactic acid into pyruvic acid in the muscular tissue which is a vital process in generating energy in the muscle cells. If there happens a damage to the cells, cells comprising particular iso enzyme of LDH is freed in the circulation. Evaluating the ratio of particular iso-enzymes would lead us to recognise definite pattern in order to help in diagnosing the diseases such as myocardial infarction, injuries to the lung, hepatic diseases, disorders of muscular tissue, various late stage carcinomas and auto-immune diseases. Various disease processes linked with elevated Human lactate de-hydrogenase (LDH) isoenzymes are as follows:

LDH 1 isoenzymes: tumor associated with germ cells.

LDH 1 > LDH 2 isoenzymes: kidney injury and Cardiac muscle necrosis, megaloblastic anemia, lysis of red cells.

LDH 3 isoenzyme: platelet damage, reactive Lymph adenopathy, lymphoma, lymphocytosis, pulmonary infarction, pulmonary infection i.e. pneumonitis, late stage advanced carcinomas, acute pancreatitis.

LDH 4 isoenzyme – different type solid tumours.

LDH 5 isoenzyme: primary hepatic/liver disorder, anoxic injury of hepatocytes, muscular damage or degeneration.

All the isoenzymes: Kidney disorders, end stage carcinomas, collagen vascular disease, severe sepsis, disseminated intravascular coagulation.

When illness or damage occurs in the tissues encompassing LDH, the cellular structure would discharge LDH within the circulation, from where it can be recognized in abnormally high levels. For an instance, if an individual has a myocardial infarction the levels of the LDH starts to increase in about twelve hours after the infarction and generally the levels might return to base line in about five to ten days. The LDH is also seems to be raised in hepatic disease, in particular type of anaemias, and in condition associated with tissue damage in excess as found in bony injury, traumatic injury, muscle destruction and severe shock. Certain types of tumours lead to an elevated levels of LDH. Moreover few patients have chronic elevation of LDH with no specific cause and without any effect.

PREPARATION

Estimation of LDH is done in blood samples. It does not require the person to be in the fasting state when the test is being done.

NORMAL RANGE

There might be variation in the values of LDH as coated as references, between lab to lab. In children the normal range of LDH might be high. But in adult population many of the labs have the value ranging till 200 units/L, although typically the value is in between 45 to 90 Units/Litre.

ABNORMAL RANGE

Total lactate dehydrogenase comprises of mixture of 5 types of iso enzymes of LDH and even though many diseases lead to elevation of LDH, diagnosing the specific disease process, each component is useful. LDH is often helpful for diagnosis. It is known that total lactate dehydrogenase might be well within the usual range but a specific iso enzyme would be elevated indicating injury to a particular tissues or organs. This can be explained by the following fact that in normal situation the component of LDH 2 within the total is higher when compared with that of LDH 1 in a given sample. But following acute myocardial injury the fraction of LDH 1 would rise beyond the fraction of LDH 2 which is termed to be "flipped LDH." It is also known that determining the total LDH might be helpful in diagnosing a particular disease. This can be explained by the following, when there is a disease infectious mononucleosis, it would lead to higher increase of LDH than that of a hepatic enzyme aspartate transaminase. But in converse infection of the liver by hepatotropic

viruses may lead to a great elevation of the hepatic enzymes like AST and ALT than that of lactate dehydrogenase.

ENZYME

It is a protein regulating the speed of a reactions involving the chemicals inside the tissues and it also increase the rate of the reaction.

ISOENZYME

These are the enzyme groups that help in same chemical reaction but they tend to have different property physically.

Since there might be variations in the LDH values because of varying conditions while collecting sample and also transporting it should be taken into account while interpreting the results. Even though lysis of the blood cells is usually identified easily and the information is notified to the labs, other processes which include lengthy refrigeration of un-centrifuged sample or centrifuge, over heating may lead to an increased level artificially. When the test is repeated this could be clarified. In the situation where the other lab features and clinical features are not present, estimating LDH iso enzyme levels would provide the best direction towards making a diagnosis. Alternative likelihood is occult tumours. But in this situation with high elevated LDH there might be raise in blood uric acid levels and if there is hepatic involvement alkaline phosphatase and gamma glutamyl transferase would be raised.

Requesting many of the blood markers for diagnosing a tumour in general is of low value. Instead of that possibly the more helpful methodology is additional thoroughly examining the patients clinically and probably few other investigations to rule out multiple myeloma.

Mainly in the elder population of patients LDH is found to be commonly elevated because of the cardiac valvular disease leading to lysis of the red cells. Above process is associated with

low levels of haptoglobulin because of binding with the free haemoglobin and commonly associated with increase in reticulocyte count and raise in indirect fraction of serum bilirubin.

Diagnosing the patient is made mostly simplified with good clinical history. Matured cells would display LDH 1 and LDH 2 iso enzymes. Above feature is seen most often in all process comprising more and more turnover of the cell in conditions like abnormal hemoglobulin, erythropoeitic inefficiency and absorption of huge blood clot.

Inflammation of the myocytes and lower level of degeneration of skeletal muscles can lead to elevated levels of LDH. Even though creatinine kinase is more often increased this is sometimes not the scenario. Easy fatigability and loss of power are the clinical features that might had gone unnoticed during eliciting history. Lactate dehydrogenase iso enzyme would show an elevated LDH 5 fraction with lower level of elevated LDH 4 iso enzyme, a type that is fundamentally cannot be distinguished due to a hepatic origin, even though a normal ALT level contends intensely not in favour of the hepatic origin. In order to clarify it more clearly tests to detect antibodies and tests for the functions of the thyroid gland is helpful. LDH levels are found to be a good marker for sickle cell anemia activity in pediatric population. In younger people with haemoglobin SS and haemoglobin C the values of serum lactate de hydrogenase (LDH) is measured and its association with vascular changes in the brain identified by transcranial Doppler scanning (TCD) is been studied. In a study all child population having Hemoglobin SS (n = 97) and Hemoglobin SC (n = 18) were undergone a transcranial Doppler study scan. Their lactate dehydrogenase values were compared with transcranial Doppler findings. It was found that there was appreciable and positive correlation. Population with haemoglobin SS was found to have higher lactate dehydrogenase levels when compared with those of haemoglobin SC disease. It showed a significant statistical difference. It was also noted that level of lactate dehydrogenase in child population with haemoglobin SS, was related considerably with level of red cells, reticulocyte, AST and

creatinine. Serum LDH values along with glycolysis considerably associated with tumor vascular endothelial growth factor A.

RED CELL DISTRIBUTION WIDTH

The greatest vital features of doing a complete blood count is that of identifying level of RBC, total leucocyte number, the percentage of different leucocytes and number of platelets. Nevertheless recent lab machineries used for complete blood count offer more features by enabling to provide more derived values[81]. Derived parameters include features of RBC and platelets. They are - Mean Corpuscular Volume, Mean Corpuscular Hemoglobin, Mean corpuscular haemoglobin concentration, Red cell distribution width, Mean platelet volume and Platelet distribution width. Above indices are very well helpful in making a diagnosis. But such indices are not used properly used since many doctors are not familiarised with that. Few of the indices are additionally included in the complete blood count results and its implication may be unknown for majority. The Red cell distribution width is one such indices.

EVALUATION OF THE RDW[82]

Inspecting the peripheral blood picture microscopically would show many red cells with different sizes that is stated as anisocytosis. This is, nevertheless, done little subjectively and could be performed most approximately. Latest automated electronic haematological machines would judge the volume of red blood cells without difficulty and more precisely, permitting an accurate value of the red cell index that gives variation in red cell volume.

The measurement is known as the 'Red Cell Volume Distribution Width' or RDW in abbreviation. This is calculated and derived as the Coefficient of Variation (CV) and is calculated by the following. Mathematically the RDW is derived using the following formula:

RDW = (Standard deviation of red cell width ÷ mean cell width) × 100

CV = Standard Deviation of red cell size ÷ MCV

	MCV Low	MCV Normal	MCV High
RDW High	Iron Deficiency Hbg Disease S/Beta Thalassaemia Hb1AC MAHA Severe anaemia of chronic disorders	Early Iron Deficiency Early B12/Folate defficency Sickle Cell Anaemia Sickle/C Disease	B12/Folate deficiency Immune Haemolytic anaemia Cold Agglutinins Alcoholism
RDW Normal	Thalassaemia trait Anaemia of Chronic Disorders Hereditary Spherocytosis Sickle Cell trait	Normal Myelodysplasia	Aplastic Anaemia

Table 3: MCV Vs RDW

Red cell distribution width is of mathematical measurement of anisocytosis. It might be of helpful in differentiating various factors leading to anaemia. But specifically in differentiating anemia of low serum iron which has high red cell distribution width and that of Thalassaemia which has normal red cell distribution width and also in differentiating anemia with Megaloblastosis which has a raised red cell distribution width and various other macrocytic anemia which might have usually normal RDW . It should be noticed that only elevated RDW are of more important and lesser values are not of significant. Sensitivity of

red cell distribution width is higher for finding abnormal feature in microcytosis than that of macrocytosis in a situation where the level of haemoglobin is on the lower range.

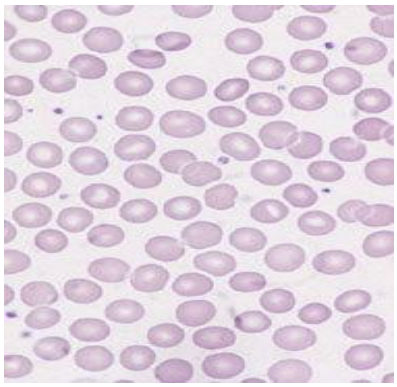


Figure 4: Normal peripheral smear film with varied size of RBC

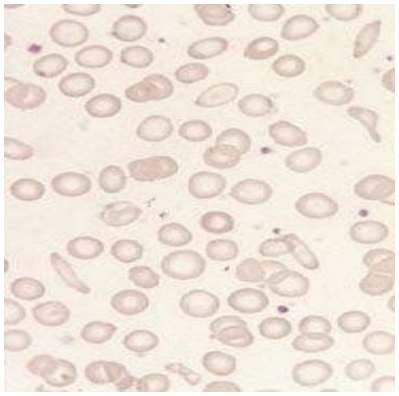


Figure 5: Red cells in moderate iron deficiency showing varied in size (anisocytosis) and shape (poikilocytosis), and also microcytosis (low average cell size) and hypochromasia (increased central pallor).

The causative factors and or characters of Anisocytosis are as follows:

Other syndromes

- Myelodysplastic syndrome

Blood abnormality

- Sideroblastic anaemia

Biochemical abnormality

- Folate deficiency

Mendelian inherited disorders

- Diamond-Blackfan anemia
- Haemoglobin Bart's
- Haemoglobin H disease

Autosomal dominantly transmitted disorders

- 4-hydroxyphenylpyruvate hydroxylase deficiency

Autosomal recessively transmitted disorders

- Beta-thalassaemia
- Congenital dys-erythropoietic anaemia type II

Nutritional disorders

- Reduced Folate levels deficiency
- Low Iron levels
- Kwashiorkor (protein deficiency)

• Low Vitamin A

• Low Vitamin B12

Iatrogenic conditions

• Blood transfusions and its consequences

Normal range of RDW in human red blood cells is 11 – 15%

RETICOUNT[83,84]

Components of the blood cells all originate from a stem present in the bone marrow. During development on multiplication these stem cells segregate as different blood cell component lines i.e erythropoiesis, granulopoiesis and thrombopoiesis. The life span of the red cells in the circulation is about 120 days. One percentage among the total red cell mass is destroyed in a day and it is replaced by production of newer cells. About 2 million red cells are formed every second. The ortho-chromatic erythroblasts expel its nucleus from the cell and produce the reticulocytic cell that later joins the peripheral blood. In about a period of 4 days removal of the endoplasmic reticulum occurs leading to development of matured red cells. In general it is noted that the reticulocyte stay inside bone marrow for four more days and one more day in the peripheries. The term ‘reticulocyte’ originates because of the web like structures present in the cells and that is evidenced by using supra vital stains like brilliant cresyl blue or methylene blue. This occurs because RNA fragments get precipitated. Reticulocyte was firstly described in 1865 by Erbs, who identified the reticulum present inside the cells with the help of picric acid. In 1881 with the help of supra vital staining, Ehrlisch identified a network called substantia reticulofilamentosa inside the cells. In 1891 Smith recognized reticulocytes to be precursors of red cells. In 1932 Heilmiyer classified the different stages

involved in the maturation of retic cells. In 1950 quantification of different stages in maturing with references was done by Seip. In 1960 reticulocytes were counted using fluorescence process with acridine orange. This was done by Kosinov & Maii. In 1983 automated retic measuring procedures with help of fluorescence technology and flow cyto metry was done by Tanki.

RETICULOCYTES AND THEIR SIGNIFICANCE

Maturation stages according to Heilmeyer	Morphological description	Quantification according to Seip (normal %)
Stage 0	Nucleolus	
Stage I	Reticulum consists of dense clots	< 0.1
Stage II	Loosely arranged reticulum	7.0
Stage III	Diffusely arranged reticulum	32.0
Stage IV	Some scattered granulae	61.0

Table 4: Stage of Maturation – by Heilmiyer

Different maturation stages more frequently are understood wrongly. Quite often stage I is expressed as an erythro blast and matured red cells as stage IV because their lower ribo nucleic acid component is undetected. The accurate analysis of stage IV is particularly imperative, because this is governed in blood peripherally.

Because there was a regular wrong interpretations of stage IV, Gelmer and Koipke gave a definition for reticulocytes: 'A reticulocyte is a red cell without nucleus, it should be having minimum 2 or greater particulates which stains blue with basophilic polyribosomal material within the cytoplasm while stained using newer methylene blue. These particle or the dot shall be at little away from the walls of the cell so that it is not been erroneously identified as

Heinzs body. Cells that have unambiguous, blue cytoplasmic granules, that might be visualised by not using fine focus is considered to be reticulocytes of maturation stage IV'.

In the year 1985 the National Committee for Clinical Laboratory Standards (NCCLS) in United states and the International Council for Standardization in Haematology (ICSH) described cell having 'single-dot' to be as matured red blood cells but not retic cells and incorporated and endorsed those to be as appraisal standard for microscopic (manual) counting and classification. Red cell regenerative process is reflected by the retic count.

In a well balanced condition more than ninety percent of the more matured stages of retic cells would be seen in the peripheries of the blood. If there is a stimulation for erythropoitic process, shift towards the early maturation stages occurs in the blood peripheries which is same that of shift towards left during granulopoietic process.

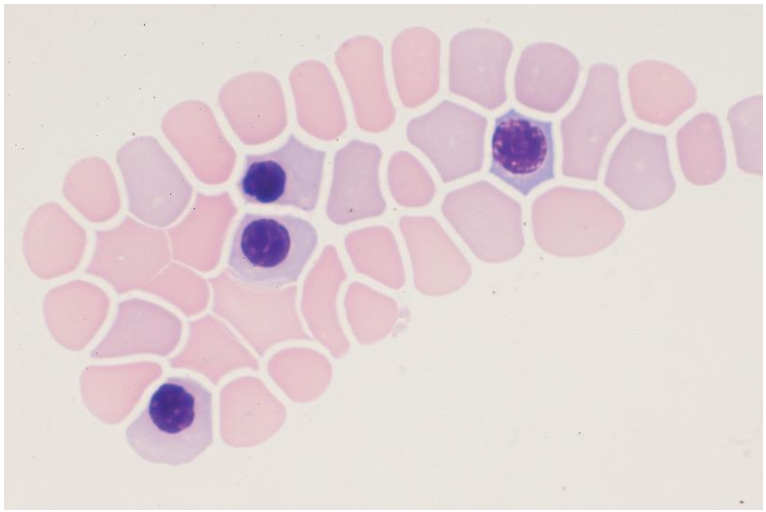


Figure 6: Polychromasia and early red cell stages in more severly haemolytic process in e auto immune haemolysis

NEED FOR DOING RETIC COUNTS

- As a preliminary test in diagnosing any anemia
- To monitor the effect of supplementation of iron, folate and B12 vitamin.
- To monitor the effect after erythropoietin injection
- Following up of hematopoietic cell transplants

SPECIMEN

EDTA blood

INVITRO STABILITY OF RETIC COUNTS[85]

Finding reticulocytes with in EDTA blood can be relied until seventy two hours of taking the blood sample. When the sample is been stored in the temperature of + 4°C or 20°C does not produce noteworthy change in the measuring results.

PROCEDURE IN COUNTING MANUALLY

Things essential for determining the reticulocyte count are supra vital stain, microscope slides and microscope. First step is to mix the specimen of whole blood along same amount of supra vital stains. Usually brilliant cresyl blue is used. Commercial preparation of the stain is there in the market in ready to use condition. The above mixture is allowed for incubation and a smear is prepared over a microscopic slides. Then smear is looked into by using a microscope through oil immersion magnifications. This gives thousand folds of magnifications. Counting

of thousand RBCs should be done. Because of thousand folds of magnifications it is equivalent to about 5 visual field. In one field there would be about two hundred RBCs. The retic count is reported in unit of per mil or in percentage.

In various literatures it have been noted that there might be higher degree or errors in manually counted. They have cited that the percentage of error might be in the range of 20 and 40. There is a standard recommendation to count thousands cells. Avoiding of More than 5 percentage of error on statistics can be done when minimum of 4000 cells are counted.

Reticulocyte count in blood (%)	Number of cells to be counted to achieve a CV of 5%
1 – 2 (reference range)	4,000
3 – 5	1,000
6 – 10	600
20 – 25	150
30 – 40	100

Table 5: Effects of numbers of counted RBCs over the error in statistics reticulocyte count. The numbers quoted denote a CV of five percentage.

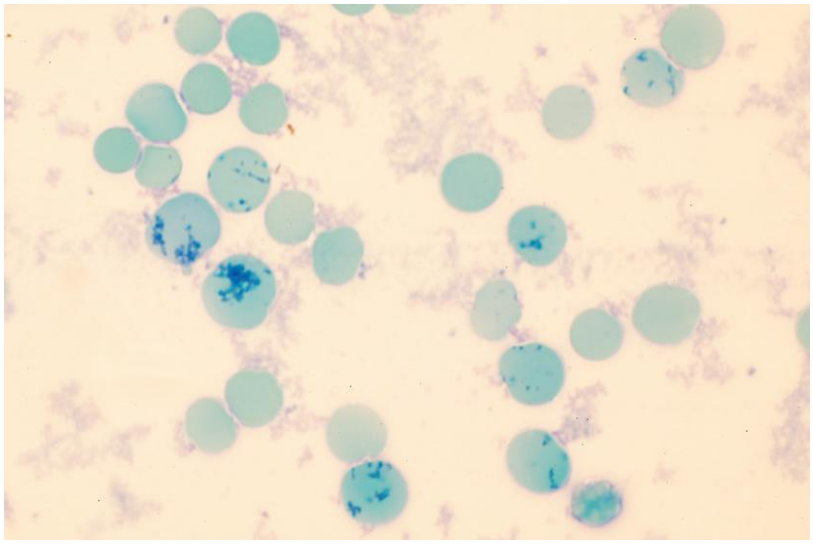


Figure 7: Reticulocyte when stained with supra vital stains

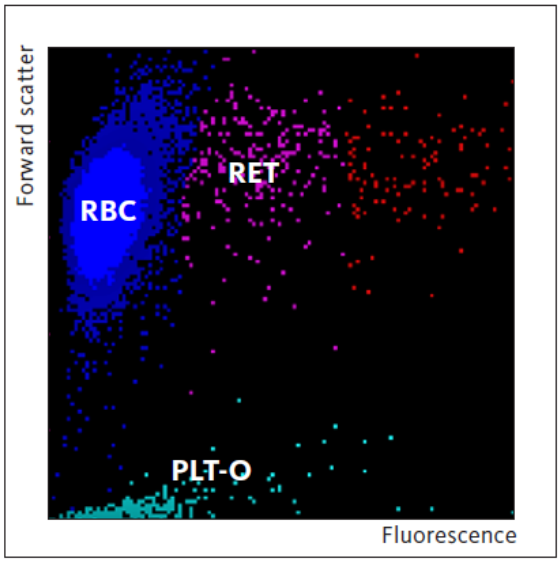
RETICULOCYTE COUNT USING AUTOMATED MACHINE

Fluorescence stains that bind Ribonucleic acid are used in staining of the blood samples in order to count reticulocte. And the counting is done using flow cytometry. Counting reiculocytes using automatic method lead to an objective threshold to classify the cell. This ensures a higher level of reproducibility in the result. About ten to thitry thousand RBCs are studied in the method. Higher counts rate and higher degrres of accuracy is obtained in this process. When comparing reticulocyte count done manually along with automated method lot of time is conserved as the result is given in lesser than a minute by later method.

Erythropoietic process and its degree of activation are identified by using retic count and its different indices of maturations. It is customary that the retic count is given as the values in percentage or as per mil of the RBC counts.

When severity of the anemia is more the analysis of the reticulocytes counts is challenging. When the severity of anemia is high moderately higher and relatively increased reticulocyte counts does not mean erythropoetic process is stronger. It could be indicative just of

shortening of RBC s life span. It would be is desirable that the absolute reticulocyte concentration is given as number rericulocyte per microlitre since capacity of the erythropoeisis is provided directly. For an instance if the calculated retic count is 20 percent and is said as elevated. Nonetheless, in severity of anaemia is more having two million RBCs, 20 percent reticulocyte count would just represent 40,000 reticulocyte per micro litre, which in the lower range of references.



RBC = mature red blood cells,
RET = reticulocytes,
PLT-O= fluorescence-optical platelets

Figure 8: Scattergram representing reticulocytes measuring channel

Relative number (reticulocytes ‰, %)	->	life span of red blood cells
Cell concentration (reticulocytes/µL)	->	erythropoietic productivity

Table 6: Comparison of reticulocyte parameters

Converting the relative count percentage into cell concentration (RET/microL):

$$\frac{\text{RET [\%] x RBC [10}^6\text{/µL]}}{100} = \text{reticulocyte concentration [10}^6\text{/µL]}$$

Reference ranges:

Relative reticulocytes:	f	0.54 – 2.02 %
	m	0.48 – 1.64 %
Reticulocyte concentration:	f	0.025 – 0.102 x 10^6/µL
	m	0.026 – 0.078 x 10^6/µL

Determining reticulocytes count with the help of fluorescence flow cytometry is said to be gold standard.

Calculating Reticulocytes index (RI)

The proportions of the reticulocytes might be elevated relatively when there is an increase in actual reticulocyte number or there is a lowering of RBC count. Corrective measure might be done with haematocrit value of the patient in reference with the normal hematocrit which is 0.45 [L/L].

The above method of correcting is advised in case of anemia.

$$RI = \frac{\text{ReT [\%] x HcT [L/L] (patients)}}{\text{0.45 [L/L] (standerd HcT)}}$$

Calculating Reticulocytes production index (RPI)

The erythropoeitic capacity and marrows productivity is evaluated with the help of reticulocyte production index. Physiologically maturing of the reticulocyte occurs inside the marrow for three days and within the peripheries for one day. In a situation where there is increased synthesis of RBCs many of the reticulocyte could be transferred into the periphery leading to the shifting of reticulocyte maturation index.

Above process causes a predominant rise in the retculocyte in the circulation which usually be a representation of an increased erythropoietic performance. This change in duration of stay of reticulocyte in the peripheries is known as shift.

Duration taken for the reiculocyte to get matured in the marrow cavity is directly related to the hematocrit. When there is a decline in the hematocrit, there would be an elevation in duration taken for maturation. The proficiency of a bone marrow is indicated by the retic count by correcting with the following hematocrit dependent factors.

Haematocrit	Retic duration in blood
45% resp. 0.450 L/L	1 day
35% 0.350 L/L	1.5 days
25% 0.250 L/L	2 days
15% 0.150 L/L	2.5 days

Table 7: Association of HcT with the maturation time of reticulocytes in periphery

$$RPI = \frac{RET\ [\%]}{RET\ maturation\ time\ in\ blood\ in\ days} \times \frac{HCT\ [L/L]\ (patient)}{0.45\ (standard\ HCT)}$$

Example:
Patient HCT= 0.25 L/L, reticulocytes = 20

$$RPI = \frac{20\ [\%]}{2} \times \frac{0.25}{0.45} = 5.5$$

RPI evaluation [2]	
Normal	1
Anaemia with adequate regeneration	> 3
Anaemia with inadequate regeneration	< 2

Table 8: Interpretating the value for RPI

IMMATURE RETICULOCYTE FRACTION (IRF)

Indications

The erythropoeitic proliferation is evaluated with immature reticulocyte fraction which might be a first indicator. The percent of immature reticulocyte fraction starts to raise with in some hour. But the reticulocyte counts raises 2 to 3 days later. When anemia due to deficient of nutrition is being treated using erythropoietin or vitamin supplementation do not show a rise in immature reticulocyte fraction would be indicative of absence of therapeutic effect. Added to this IRF would assist in classifying anemia as hypoproliferative, normoproliferative or hyperproliferative anemia. Along with immature reticulocyte fraction the reticulocyte counts had shown to helpful to monitor marrow efficiency and also following up of stem cells

transplantation. Successfully transplanted patients with marrow stem cells about 80 percentage of subjects the Immature Reticulocyte Fraction values attains the 5 percent target in early when compared with granulocyte, classical limit of its count being 0.5 x 109 granulocyte per litre. Added to the routine reticulocytes count, fluorescent flow cytometry process helps us to classify reticulocyte as 3 stages. Those stages of maturing are been described according to its ribonucleic acid contents - using an analyzer: fluorescence intensity.

Reticulocyte maturation

LFR	MFR	HFR
Low Fluorescence Reticulocytes	Medium Fluorescence Reticulocytes	High Fluorescence Reticulocytes
Little RNA	More RNA	High level of RNA
Mature reticulocytes	Semi-mature reticulocytes	Immature reticulocytes
Reference range: 86.5 - 98.5%	Reference range: 1.5 - 11.3%	Reference range: 0 - 1.4%

Table 9: Maturation stages of reticulocytes

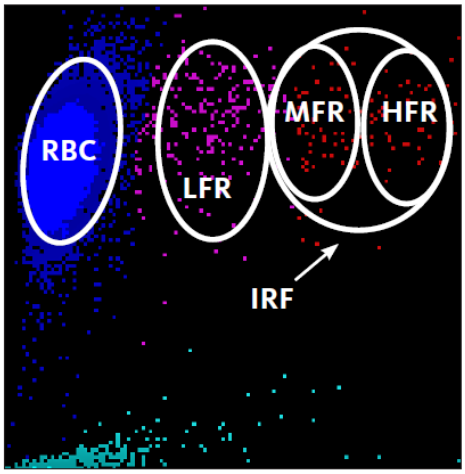


Figure 9: Scatter- gram of the reticulocytes channal

Immature reticulocyte fraction is an addition of MFR and HFR that is immature reticulocyte, and refered as reticulocytes maturation index.

Immature reticulocyte fraction = MFR + HFR

Referrence ranges

Immature reticulocyte fraction RF: f 1.1 – 15.9 %

m 1.5 – 13.7 %

Invitro stability of IRF

Six hour.

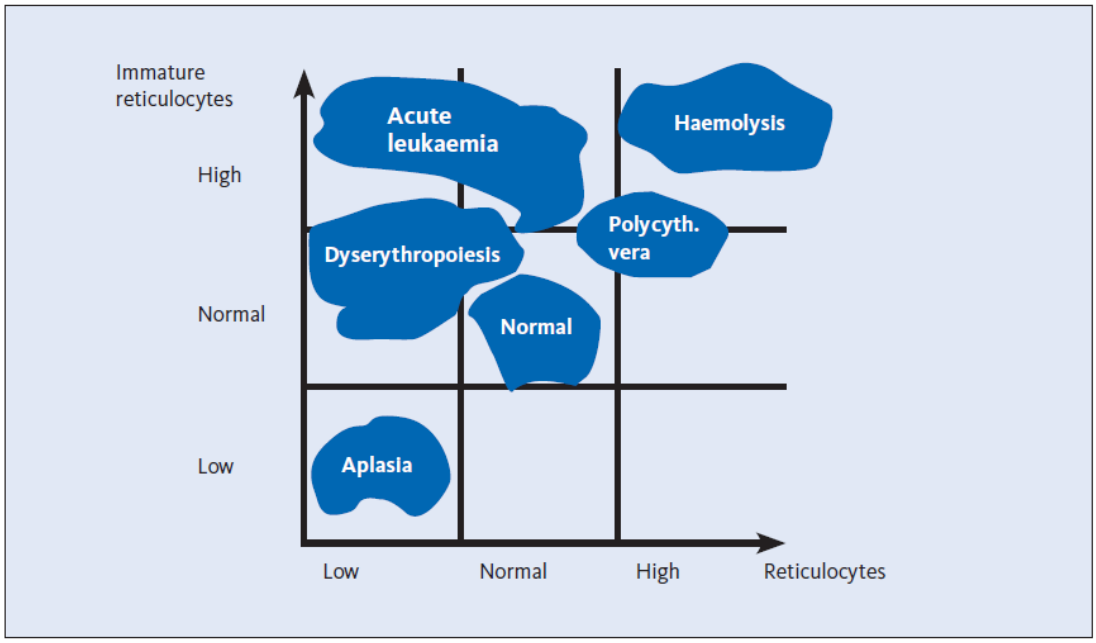


Figure10: Clinical use of IRF

RET-H*e*

Automated counters determine red blood cells, haemoglobin, reiculocyte, immature reticulocyte fraction, red cell volume which are quantitative parameters. But qualitatively it can be assed with Reticulocyte Hemoglobin equivalent. Reticulocyte haemoglobin equivalent offers haemoglobin values in the newly synthesised RBC s. This would offer a real time and thus offers real-time informations on ferritin supplied to the erythropoietic process. These are specifically helpful to find the difference among the anemia of low serum iron and that of anemia due to chronic diseases. In description it is helpful in finding the difference between real iron deficient state and sate of ‘functional iron deficiency i.e problem associated with mobilising and utilising iron.

Determining reticulocyte haemoglobin equivalent could be done on a haematology analyzer along regular peripheral smear parameter. Reticulocyte haemoglobin equivalent is not an acute phase reactant as against serum feritin and transferin which rises during acute infection or inflammation.

NORMAL HAEMOPOIETIC SYSTEM AND HAEMOPOIESIS

Sites of blood formation[86]

During the course of development from embryonic to adult life different anatomic sites are involved in formation of blood cells. First in early embryonic life embryos yolk sac starts producing blood cells. Later blood cells are being produced in the liver and to a little extent spleen is also involved. During the course of fetal development between second and seventh month of gestation these organs become the major sites of production of blood cells. Bone marrow surpases these sites of blood production in later part of gestation and become most important and only site after delivery. Despites its production from bone marrow in adult life,

the lymphocyte production also occurs in other organs. Soon after delivery in newborn production of haemopoietic cells are present in all the cavities of the bones, but with advancing age the upper shafts of the Humerus, Femur, Pelvis Skull, Spine and bones of the thorax are involved. Haematopoietic tissue in adults comprises about one to two litres in total volume.

Haematopoietic stem cells (HSC) which have the potency to develop into cells of different lineages are present in large numbers in the bone marrow. Committed lymphoid or myeloid (common lymphoid and myeloid progenitors, respectively) are developed from Multipotent HSC s. Cells like neutrophils, mast cells, megakaryocytes, erythrocytes and monocytes, and also cells like osteoclasts which are not directly related to the haematopoietic system are developed from myeloid lineage of cells. Cells like natural killer (NK) cells, T cells and B cells are developed from lymphoid lineage cells.

NORMAL BONE MARROW STRUCTURE

The trabeculae of bone present within the cavity of the bone has interspersed red marrow. Marrow also in addition contains specialized connective tissue cells, reticulin fibrils, blood vessels, fat cells, nerves and macrophages along with cells of lymphoid and myeloid series. Fine reticulin fibrils present as the component within the marrow ensures a supportive frame work for the other components of bone marrow. Adventitial reticular cells produce these reticulin fibrils that stretch between the endosteum of the bony trabeculae and the vascular sinusoids. Sinusoids contain relatively large lumens which are lined by endothelial cells of single layer through which arteriolar blood passes through. This is the site where entry of newly formed blood cells into the circulation occurs .Nearly half of the extravascular volume of the red marrow is made of fat cells and yellow bone marrows which present in the most

distal parts of the long bones nearly all of them are fat cells. Since fat cell distribution is mostly irregular in red bone marrow, while performing bone marrow specimen adequate sample size is needed to find the haemopoietic tissue cellularity.

BONE MARROW BIOPSY:[86,87]

Bone marrow aspiration has the advantage that the material obtained from the aspiration can be used to prepare the smear immediately and can be examined. It has superior morphological details than that obtained in histological sections from core biopsy material acquired from trephine biopsy. For knowing about the cellularity of haematopoietic elements and also to diagnose certain diseases like neoplastic cells or fibrotic material bone marrow trephine biopsy is more advantage than aspiration alone. This is because the above materials may not be freed from the marrow cavity by suction. From the above discussion we know that we have more additive details when both are done, so that the combined data is of immense diagnostic value than when either of them is used alone.

NEEDLE ASPIRATION BIOPSY OF THE BONE MARROW

The anatomic sites such as sternum, iliac crest or anterior or posterior iliac spines are the locations from where we can extract a satisfactory bone marrow sample, But since there is a danger of injuring a major blood vessel or the right atrium because of misdirected needle during the procedure at the sternal site it is now not the favoured site[88].

The skin over the selected site of aspiration is cleansed with antiseptic solution which may be either 70% alcohol or 0.5% chlorhexidine. Then 2% lignocaine is loaded in a syringe and infilterated to the skin,subcutaneous tissue and periosteum overlying the site selected for the

puncture. Then bone marrow aspiration needle, with a boring movement is passed into the cavity of ilium perpendicularly over the posterior superior iliac spine. As soon as we know that the needle has passed the bone, stillete is removed and 2 or 5 ml syringe which fits well is used to suck up the marrow material as such about 0.3ml of marrow material is obtained. This specimen has the variable amount of blood mixed within the marrow material. This would be a simple procedure where complications occur rarely except for sternal site puncture.[89,90]

Under the anatomic site of posterior superior iliac spine there lies a large bone marrow containing area and from where relatively large quantities of marrow can be aspirated. Position needed for the procedure over the posterior iliac spine is a prone position or lying on the sides. Since the patient is in lying position while the puncture is done it has the advantage over the sternal site that the patient will not visualize the procedure.

In patients who are all obese where there will be difficulty in locating the iliac spine computer tomography guidance is used for performing the procedure.[91]

Bone marrow films

From the specimen obtained from the aspiration a single drop is delivered on to slides about 1 cm from one end. This drop should rapidly spread out over the line of contact. Another slide is used as the spreader with which the single drop of blood is spreaded along the slide using a steady movement of the spreader slide with the hand. An important step is that the slide used as a spreader must not be lifted off till the last bit of drop of blood is spreaded over the slide. When a suitable sized drop was used the length of the film would be of 3 cms. We should make sure that it is important that the film of blood stop short about at least 1 cm before the end of the slide. The irregular marrow fragments usually adhere to the slide and most of them will be left behind. Then make films 3 – 5 cms in length, with the marrows fragment and the

rest of the sample using a glass spread with levelled edges. Wideness of the spreader should be below 2 centimeters.

Bone marrow smear films are fixed and Romanovsky dye is utilized for staining purpose as that of using in peripheral blood smears. Few other technicians or pathologist add the aspirated material in a tube containing anticoagulant and later they prepare smear after coming back to the laboratory. By changing the certain maneuvering technicque of the spreader slide like its speed, pressure and also the angle at which it is held it is possible to alter the film thickness. The angle of the spreader slide can be adjusted according the specimen obtained to get a good quality smear. That is if the sample specimen is obtained from polycythemic blood the spreader angle should be narrow and when it is from anemic blood spreader angle should be of wider angel so that in a sample derived from polycythemic blood the angle between the spreader and slide should be narrower while in patients with anaemic blood, the angle should be wider to provide an suitable sample. Microscopic view of the smear should show some overlap of red cells in most areas all over the films length to say it as an ideal smear. We should be able easily identify the leucocytes all over at most part of the film. There would be uneven distribution of leucocytes and that the monocytes and other large leucocytes would be moved to the ends and the side of the smear if it has been poorly prepared. If a proper slide is not used and the slide has dust and if it is greasy it would produce an uneven stripy film with patchy spots.

Manual Staining of Marrow films[92,93]

Bone marrow aspirate smears are most preferably stained by Wright-Giemsa stains. It is a staining solution which is methanol based. This type of stain mostly takes a longer contact time for good and acceptable staining of the bone marrow smear. This type of stain requires a

buffer step. Even though this stain does not require a separate fixation step because it is methanol based stain, we tend to desire to do fixation since it diminishes water artifact which tend to occur in humid days and also with aged stains.

When the smears are stained by dip method, the smears are usually fixed by dipping into methanol and then it is stained with Wrights–giemsa stain by placing the smear in that stains for about 10-15 minutes. Next step the smear is placed in a mixture of stain and a phosphate buffer with ph of 6.8 and given time about 20-30 minutes to stain. As soon as staining occurs the smears are given a fast rinse with distilled water. Then it is kept for air dry and then mounting or cover slipping done.

If the bone marrow smears are stained with staining racks, the slide with marrow particles or cover slips, they are swamped enough with adequate stains so as slides are covered and staining process continues over a duration of 10-15 minutes. In next step a buffer with a ph 6.8 is prudently added avoiding overflow then softly mixed by blowing till there appears a green metallic sheen. Distilled water is used to thoroughly rinse after it is made to stand for period of 20-30 minute. Before they are mounted, cover slips or slides should be air dried.

When highly cellular marrow is expected the staining times shall be increased. But caution should be taken when rack staining method is used. Disproportionate precipitation occurs when prolonged time is used because of vaporization of the stain. This can be prevented or compensated by topping up of the stain as well as the buffer, however increased rinse time must be done.

Appropriately stained smears with Wrights and Wright Giemsa stain shall show accurate and clearly visible granules of the nucleus and also cytoplasm. Variations in the quality of stain is noticed in different batches and also it is reliant on quality control of the producers, method of storage and also the shipping environment. It is prudent to have an isolated storage of Wrights stain to stain the bone marrow staining which is stocked for duration of at least 6 months before use. Wrights stain is compared to a good wine because it shows fine quality and clarity of final stain as it gets older.

Cellular component	Colour
Nuclei	
Nucleoli	Light blue
Chromatin	Purple
Cytoplasm	
Erythroblast	Dark blue
Reticulocyte	Grey Blue
Erythrocyte	Dark pink
Lymphocyte	Blue
Metamyelocyte	Pink
Monocyte	Grey-blue
Myelocyte	Pink
Neutrophil	Pink/orange
Promyelocyte	Blue
Basophil	Blue
Granules	
Promyelocyte (primary granules)	Red or purple
Eosinophil	Red-orange
Basophil	Purple black
Neutrophil	Purple
Toxic granules	Dark blue
Platelet	Purple

Table 10a: Color taken up by different cellular components and inclusions

Other inclusions	
Auer body	Purple
Cabot ring	Purple
Howell–Jolly body	Purple
Döhle body	Light blue

Table 10b: Color taken up by different cellular components and inclusions

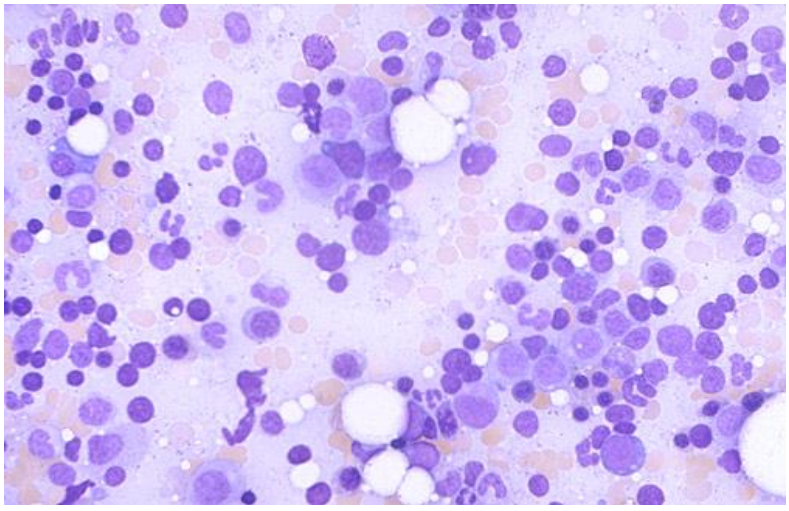


Figure 11: Normal bone marrow picture

87 94

Cells	95% Range	Mean	Mean
Myeloblasts	0–3	0.4	1.4
Promyelocytes	3–12	13.7 c	7.8
Myelocytes (neutrophil)	2–13	–	7.6
Metamyelocytes	2–6	–	4.1
Neutrophils	22–46	35.5	32.1 M; 37.4 F
Myelocytes (eosinophil)	0–3	1.6	1.3
Eosinophils	0.3–4	1.7	2.2
Basophils	0–0.5	0.2	0.1
Lymphocytes	5–20	16.1	13.1
Monocytes	0–3	2.5	1.3
Plasma cells	0–3.5	1.9	0.6
Erythroblasts	5–35	23.5	28.1 M ; 22.5 F
Megakaryocytes	0–2		0.5
Macrophages	0–2	2	0.4

Table 11: Cell composition of adult bone marrow aspirate

Figure 12: Klima Needle

The needle is advanced into the marrow cavity with the stylet locked in place. The stylet is then removed and a syinge used to aspirate the marrow.

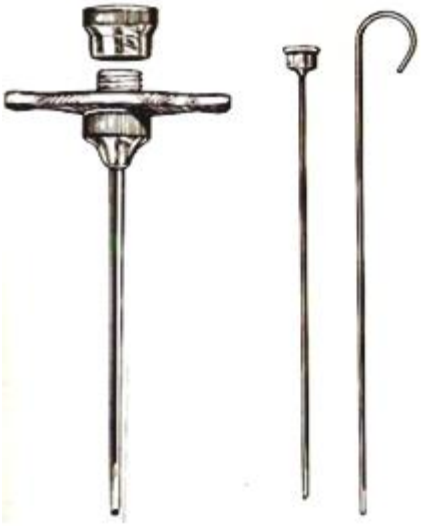


Figure 13: Jamshidi Needle

The stylet is in place for advancement through soft tissue and cortex. On reaching marrow, the needle is advanced without the stylet to create a core sample. The probe (hooked) is used to expel the sample into formalin.

Figure 14: Bone marrow aspiration

Diagnostic indications for upper gastrointestinal

The following conditions are the indication for upper GI scopy

1. When patients present with isolated dysphagia and/or odynophagia and history and examination reveals or suspicious of esophageal origin but not pre-esophageal origin. When pre-esophageal cause is suspected an thorough ENT examination and work up is needed.[95,96]

2. In patients presenting with persistent nausea or vomiting presenting for greater than 48 hours, after excluding acute intestinal obstruction and other non gastro intestinal cause.

3. It is advised in patients with dyspepsia who are all aged more than 45 years with or without signs and symptoms such as dysphagia, weight loss, anemia or other warning symptoms. In patients with age less than 45 years it is advised for suspecting H.pylori infection and treatment has failed to improve the symptoms or the symptoms relapse.[97,98]

4. It is advised for evaluation of anemia of long duration and or lower iron storage state when there is suspicion of an upper GI cause.[99,100]

5. Upper GI scopy is advised strongly as preliminary line of investigation in acute intestinal bleed which probably comes from the upper gastrointestinal tract (haematemesis or melena). It should be make sure that endoscopy is performed rapidly and in any event not exceeding 24 hours after the episode of bleeding. Therapeutic procedure needed can be performed at the same time during the scopy. Scopy shall be repeated when there is persistence of bleed or when the previous scopy or colonoscopy was inconclusive.[101]

6. Gastro-oesophageal reflux disease in patients aged more than 50 years or with warning signs of bleeding, dysphagia, weight loss or anemia or if there is no relief of symptoms after adequate treatment or relapse of symptoms when the treatment is withdrawn. Upper gastrointestinal endoscopy is indicated, after non- gastrointestinal origin has been eliminated, if there are atypical symptoms which may be related to gastrooesophageal reflux (nocturnal

cough, asthma, pain mimicking angina, hoarseness, burning sensation in the pharynx, ear pain).[102]

7. Barrett's oesophagus - To establish a diagnosis of barretts esophagus we need to do endoscopy as well as biopsy which would show dysplasia. Second biopsy is needed to be done 2-3 months after initial one and in the intervening time treatment with antisecretory drugs should be given. Regular follow up and repeated endoscopic monitoring is mandatory depending on the degree of dysplasia and cancer risk of the patient. When there is intestinal metaplasia without dysplasia repeat endoscopy along with multiple biopsies every two to three years is necessary. In patients with low grade dysplasia in biopsy we need to perform endoscopy every six months for one year and then every year. Monitoring can be stopped if there is high grade dysplasia or when it seems improbable that continued monitoring will increase survival.[103]

8. Peptic ulcer: The following are the patient population in those with typical or atypical symptoms of ulcers, upper gastrointestinal scopy is advised as the first choice

• in all subjects when their age is beyond 45 years with typical or atypical ulcers;

• in subjects when their age is below 45 years who has warning signs or symptoms or who has H.pylori positive status or treatment failure.

Multiple biopsies would be done consistently in patients with gastric ulcers. A control endoscopy might be done as part of follow-up of a gastric ulcer. When the patient is of age 45 years, persistence of symptoms even after good treatment, if there is any difficulty in identification of biopsy specimen or the earlier appearance in endoscopy is not usual. In patients with drug induced ulcers UGI scopy is advised when there is persistence of symptoms even after stopping the drugs or if there is a clinical situation the drug could not be withdrawn[97,98].

9. Upper gastrointestinal endoscopy is advised as a diagnostic procedure when portal hypertension is a diagnostic possibility and also to search for varices in esophagus and stomach in the patients who has cirrhosis of liver. When the initial endoscopy does not show varices in patients with cirrhosis regular follow up every two years with endoscopy is suggested. Check endoscopy is advised in patient who has undergone endoscopic treatment for esophageal varices.[104]

10. Duodenal biopsy[105]: Following circumstances warrant to undergo a duodenal biopsy along with UI scopy

• in patients with iron-deficiency anaemia in whom a specific cause can not be made out.

• in subjects having low folate levels, this is usually combined with gastric biopsy

• in subjects with deficiency of other nutritional factors

• in patients who are under evaluation for chronic diarrhoea;

• in patients with dermatitis herpetiformis;

• to asses the therapeutic response to a gluten- free diet in coeliac disease;

• when a specific parasite is strongly suspected to be infested and stool examination is negative for the specific organism (lambliasis, strongyloidiasis).

THE ENDOSCOPY PROCEDURE

Patient planned for an upper gastroduodenal endoscopy, informed consent is obtained, are kept nil by oral for about 8 hours. The patient is made to lie on his left lateral side for the procedure to be performed. It would take about 20 to 30 minutes for the procedure to be finished. Time depends on whether biopsy is taken or not[106,107]. First topical lignocaine is applied to the throat through spray technique or else patient is made to gargle with the local anaesthetic solution. This makes the throat to feel numb. Then between the teeth a plastic mouth guard is placed so that it avoids any damages being done to the dentition as well as to

the instrument. The endoscope also named to be gastroscope is a flexible tube that has the size of finger. Source of light and a lens is present in the scope so as to enable the scopist to visualise the epithelium of the upper intestines mostly in a television screen. The endoscope has a lens and a light source that allows the endoscopist to see the inner lining of the upper gastrointestinal (GI) tract, usually on a TV monitor. The endosope is passed oraly between the teeth protected by the mouth guard and patient is instructed to make swallow movement as soon as the tip of the scope reaches the oropharynx enabling smooth passage into the esophagus. In overtly anxious subjects mild sedation can be offered.

Another method is to pass a thin scope through the nasal cavity to enter the esophagus. This is called as trans nasal approach[108]. Topical anaesthetic agent is applied to the nostrils before the procedure. Along the scope air is smoothly presented into the intestines so as to dilate the oesophagus, stomach, and small and large bowel so that the scope is able to pass via those regions and improves the visualisation the scopist. Mild discomfort would be experienced by the subject while injecting air into the stomach and intestine.

Patient may feel fullness of the stomach which will be relieved after belching. Normal breathing is not interfered with the procedure. Patients are instructed to be relaxed and asked to take slow and deep breaths during the procedure.

RECOVERY FROM ENDOSCOPY

Patients are allowed to stay for about an hour or less for observation. This is usually for action of the sedative drugs to wear off. If sedative drug is been used patient may feel tired for short duration and also have mild concentrating difficulty. Such patients are advised not to drive vehicle or resume their work on the day of procedure. After the procedure patient might have minimal sore throat, but patient is able to tolerate oral feeds soon after the

procedure. They might also have mild bloating sensation of the abdomen if air has passed to the intestine.

ENDOSCOPY COMPLICATIONS

Complication of Upper endoscopy is very minimal or almost negligible because it is a simple procedure. Following are few complications while doing an endoscopy

- Inhalation of food particles or gastric secretions.
- Reactions to the sedative medications.
- Biopsy site bleed or bleed from site o where polyp is removed. Though these Bleed are smaller in amount and usually gets arrested by itself or able to control with ease.
- Injury or rupture of the esophagus, stomach or duodenum, but usually rare.

Patients are advised to report immediately if they develop the following symptoms:

- Intense pain abdomen
- Abdomen distention
- Recurrent and severe vomiting
- Fever
- Finding difficult to swallow or intense painful throat
- Feel of crunch below the skin over the neck area.

DUODENAL BIOPSY

Tubular structure which is present as continuation between stomach and the cloaca develops intestines. The initial portion of this tubular structure gives rise to the portion of the intestine extending between distal part of duodenum and proximal part of the ileum. The distal part of the ileum and initial 2/3 rd of the transverse colon is developed from the little distal part of the tubular structure. With the growth of the embryo the tubule of endodermis gives rise to the epithelial lining. Further during development of the embryo at 9 to 10 week of gestation stratified squamous type of lining is converted into simple columnar typo of epithelium. Villus forming process starts at ninth week. About five days prior to delivery the epithelial lining differentiates morphologically into its finale structure. About during the middle of the gestational age initial stage of lymphatic cells begins to appear. In about sixteenth week of gestational age aggregation of T-cell and B-cell occurs as aggregates of T and B cells form primary Peyers patch. By the 19th week of gestation fully developed peyers patches appear.

HOW TO EVALUATE SMALL INTESTINAL BIOSY

In assessing any biopsy tissue obtained from the duodenum clinico-pathological relation is needed to diagnose a disease. Adequate clinical data should be provided to the pathology department by the treating physician in order to make good assessment. Pathologist should go read through the request form in order to determine the significant feature of the patient clinically and also to clearly know about the sites from where the specimen was taken.

Data to be furnished to the pathology lab are as follow:

1. Age and sex of the patient
2. Signs and symptoms, site of the biopsy, endoscopic findings, radiological findings
3. Clinical diagnosis or impression
4. Medical and surgical history

5. History of taking drugs or alcohol

6. History of immunosuppression

7. Findings of previous biopsies

Routinely taking biopsies from the duodenum for whatever the scopy indication might be or whatever might be the appearance of the epithelium macroscopically is a bit in divisive[109]. But on the other side routinely taking biopsy from the duodenum could give an unanticipated finding pathologically, that might have chief association clinically. But the hazard of performing anaesthesia which could have been averted and also problems of procedure in getting specimen from seemingly appearing regular mucosal surface is in upsurge.

Over all, the cost benefit ratios might not be in favourable position. Nonetheless, it has been noted in most of the trials that unanticipated pathological features are seen in the specimen taken routinely from the duodenum[110,111]. One research done with cohort of children disclosed that taking tissues from the duodenum yielded abnormal pathological features in about 17% of the children[110]. Unpredicted pathological features are found to be reported in various research papers like giardiasis, minimally inflamed mucosa chronically in the lammina propria, more number of plasma cells in the mucosal lining in the specimen obtained from the stomach related H.pylori colonisation. In one research upsurge is noted on the intraepithelial lymphocytes (IEL) counts, without a positive anti-endomysial antibody and also be related to the primary symptomatology. Above research paper stated that more frequent i.e. in more than half of the subjects the reason for endoscopic procedure was to evaluate anemias. This paper gave statistical significance saying that normally appearing mucosal surface macroscopically may show findings of certain features pathologically[110]. It can be said the assessment of the specimen obtained routinely from the duodenum might be of valued procedure in aiding and diagnosing a disease pathologically which was not suspected in subjects with no symptoms or else whom do not have typical symptoms[110,111].

The numbers of biopsies taken simultaneously from the duodenum were inconstant and also the sites from were taken. Few researchers recommend to take at least 3 samples of duodenal tissues distally [112]. Due to the involvement of mucosa is restricted and are irregular, many number of samples should be taken to lessen the chance of under-diagnosis and or misdiagnosis[113]. This type of situation arises in many conditions. Destruction of the duodenum brought about through glutens appears to begin in the bulbs of the duodenum. Then later it slowly progress to involve the other duodenal parts and initial jejunal portion. Subsequently, the position and the quantity of the tissue specimen would play a significant role in making an exact disease process of GSE[114]. In Gluten sensitive enteropathy pathology of the duodenum might be in patches that too particularly in childhood population[114] and also atrophied villi might be seen adjacent with or concur the atrophied mucosa or the mucosal surface without any abnormality. The mucosal surface of the bulbous part of the duodenum alone is diseased when the specimens were stained with special stains. Analysing with histo-chemical staining are mostly carried out to identify mico-organisms and the component of the cells.

1. Periodic acid Schiffs stains for identifying macrophage in Whipple's diseases, fungal infection, metaplasias of mucosa of the stomach and duodenal inflammation and also the stability of the brush-border.

2. Di-astase periodic acid Schiffs or methenamine silver stain are used in Cryptococcal neoformens.

3. Wade-Fite, Ziehl Nielsens or Aouramine stains to stain mycobacteriums.

4. Warthin Starrys stains for microsporidium.

5. Iron hematoxylin countar stains in tri-chrome techniques, Giemsas stain, toluedine blue for Giardia.

6. Immuno-peroxidase stain for cytomegalo-virus. Immuno-histo-chemistry is applied to see CD-3, CD-4 and CD-8 in Gluten sensitive enteropathy or refractory sprues.

INTERPRETATING TISSUE BIOPSY OF DUODENUM

EVALUATING THE ARCHITECTURES

Visualising with Low power magnifying lens of the microscope would provide evidence about the gross look and the structure of the mucosal surface of the duodenum. A well oriented biopsied tissue must be having at least four to five sequentially long, villous structures which have distension beginning in the basal portion extending to its tips. The architectural structure of the villi might show varied range of alteration in the morphology among different cohorts. Morphologicaly Villi comprises various shapes such as fingers like, leaves like, tongue like or ridges. Duodenal mucosal surface commonly has branching villous and villous with glued tips or the mixer of the above two.

Morphologically the villi structure varies with location of the patient in geography. Villous structure seen at temperate area might be of long and finger like structures, but in regions of tropics villous might be alike leaves or ridges in the proximal portion and alike finger in the distal part[115]. Normally V:C ratios appears approximately between 3 to 5:I. When there is shortened and blunted villous structures causing flattened mucosa it is called as atrophied villi. This atrophied villi are graded according to the V : C ratio. It is called total when it is between 0 to 1 : I or else in-complete when it is between 1 to 4 : I [116]. Atrophying of villi is said to be not of any specific pathology but could be of reaction to diverse injury. The pathogenetic mechanism involved could either be connected to the elongated crypts caused

by hyper regeneration in the mitotic activity of cellularity of the crypts as seen in gluten sensitive enteropathy or it can occur because of hypo renewal of cell which means low mitosis activity in the cells producing condensed cryptic structures that might be seen in patients who are starved or those are on TPN[117]. Multiplication of the crypts cellular structure is initiated by activation of T cells. As a result of which active CD – 4 T cell are responsible in maintaining the mucosa with normal structure[117].

Tissue from mucosa of the duodenum gives rise to particular issues in arriving at a decision. The villous structures are short and thick in duodenum when compared with that of jejuna mucosa specifically in the mucosa over the Brunner gland[118] or lymph cell follicle. Henceforth diagnosing atrophied villi in bulbous part of duodenum is tough[113]. In whipples diseases and in MAC infection blunting and shortening of villous structures occurs or seen as atrophied because penetration with macrophage cells or densely infiltrating plasma cell and centrocytes alike lymphocytic cells or pleo-morphic lymphocytic cells.

In above diseases, the superimposing epithelial layer would be normal, cryptic structures are exempted also the Intraepithelial lymphocyte counts are in usual range. Cryptic hyperplasias might be due to repairing effect or re-creating process[119]. It is categorised by lengthening of the cryptic structure, reduced C : V ratios and more mitotic activity because of enlargement in the proliferating section of the crypts from its base extending through the entire height.

Evaluating cryptic hyperplasia in disease of gluten sensitive enteropathy is usually tough, particularly while there is preservation of the structure of the villi. K i 67 indeces[120] would be supreme marker for sensitivity to asses cryptic hyperplasia. Epithelial surfaces constitute of columnar cell of absorbing type called entero-cytes and cells which produces mucous secretions called the goblets cell. The enterocyte cells characteristically have the nucleus at the base and are regularly allied. Periodic acid schiffs stain positivity is seen on the lumen

surface of the entero-cytes. Enterocytic cells have the goblets cell dispersed in between them. Alcain blue positivity is seen because of mucain in the goblet cell. Paneths cell of the crypt's are indicated by brightly esinophilic, supra nuclear granular structures in the cytoplasm.

Cryptic endocrine cells are seen singly or in aggregated form. Distinct from the paneths cell endocrine cells are seen at the base of the cells i.e below the nucleus. Places among the individual villous present over epithelial surfaces should be in between the villi on the surface epithelium have to be prudently checked to discount the existence of Giardia.

Cautiously inspecting the epithelial upper layer particularly over villous tip is mandatory since few of the research authors had said that C D 3 lymphocytic intraepithelial cells are distributed mostly on the top, suggesting gluten sensitive enteropathy[119,121,122,123].

In addition, plentiful protozoas which infects small intestine like microsporidium, cyclosporum and isosporum preferentially present at the enterocyte in the villous tip and the epithelial surface might be showing disruption of the architecture and disintegration focally[124]. They might display non specific change of degeneration like cytoplasmic vacuolization or gastric-metaplasia.

The lamina propria frequently contains plasma cells and lymphocytic cells with very infrequent appearence of eosinophills and macrophage cells. Secretion of immunoglobulin A into the lumen of the intestine is by the plasma cell present in the lamina propria. Mucosa associated lymphoid nodule are spreaded all over the mucosal surface and accumulate into visible Peyer's patches.

An increase in thickness of the mucosa can be a consequence of edema and a raise in the count of various inflammatory cells[125]. The bulk of the lamina propria is enlarged and would be doubled when comparing to that of normal mucosal layer in lesions of flat destruction seen in gluten sensitive enteropathy[125].

In Biopsies obtained from duodenum of subjects having a disease of dermatitis-herpatiformis the escalating distension seen in lamina-propria ensues with permeation of inflammatory cells. Architectural change in the mucosa advances from type 1 through type 3 finally becoming even. Undoubtedly, edema and infiltrating neutrophils into the lamina-propria are primary features found to seen along with enlarging crypts. This might be isolated phenomenon indicating changes in the architecture[125]. An acute inflammatory change could be much strong which might imitate as of peptic ulcer[119]. The elevation of neutrophill counts is reliant over the doses of the gluten exposure[125].

Few of the times eosinophils are noticeable and found infiltrating epithelial layer in gluten sensitive enteropathy[119] and with allergic conditions associated foods[126,127]. The lamina propria might be populated by Cryptococcal organism or by enlarged macrophage infiltration as of in infections with mycobacterium infection, leischmaniasis or Whipples diseases.

Inclusion bodies associated with viral infection like cyto-megalo viruses are seen within the cells of endothelium, fibroblastic stromal cells, in brunner glands and also in the smooth muscular cell.

MATERIALS AND METHODS

This is a prospective and descriptive study which was done over a period of 12 months on 50 patients with age ≥15 years who were admitted with macrocytic anemia in PSG hospitals, Coimbatore.

Macrocytic anemia was diagnosed in patients with

1. A mean red blood corpuscular volume >95 fl and
2. Anemia with a hemoglobin of
    a. < 13 gm/dl in male patient
    b. < 12 gm/dl in female patient

Inclusion Criteria

Patients above the age of 15 years with macrocytic anemia.

Exclusion Criteria

Patients with decompensated liver disease, chronic kidney disease, Hemolytic anemia, Hemorrhagic disease.

Post splenectomy and pregnant patients.

Detailed history was obtained from the patients. Full and methodical clinical examination was done in all patients. Complete information was obtained regarding alcohol intake, drug intake, thyroid disorder and other comorbid illness. All patients were investigated with a complete haemogram which includes estimating hemoglobin level, red blood cell indices

(MCV, MCH, MCHC), red cell distribution width (RDW), total leucocyte count, differential leucocyte count, platelet count, reticulocyte count, and examination of peripheral smear.

Whenever the patients showed the features of megaloblastosis like occurrence of hypersegmented neutrophils or macro ovalocytes bone marrow aspiration and biopsy was done after obtaining the consent. Liver function tests were done in patients who presented with icterus. Serum TSH was done to rule out Thyroid dysfunction.

Patients fasting vitamin B_{12} and folic acid were measured.

An upper gastrointestinal endoscopy with biopsies from deep duodenum was done in patients with megaloblastic anemia who consented for the procedure.

RESULTS

In our study population of fifty patients presenting with macrocytic anaemia 34 were male comprising about 68% and 16 were female comprising about 32%. The mean age of male and female were **53.15 ± 15.89** and **54.37 ± 16.58** respectively. Majority of the population belong to the age group **above 40 years**, both in males and females.

Sex	n	Percentage
M	34	68%
F	16	32%

Table12: Sex distribution

Sex	M	F
Age 15 – 25	3	2
Age 26 - 40	4	1
Age 41 - 60	17	7
Age > 60	10	6
Percentage	68 %	32%

Table 13: Age distribution

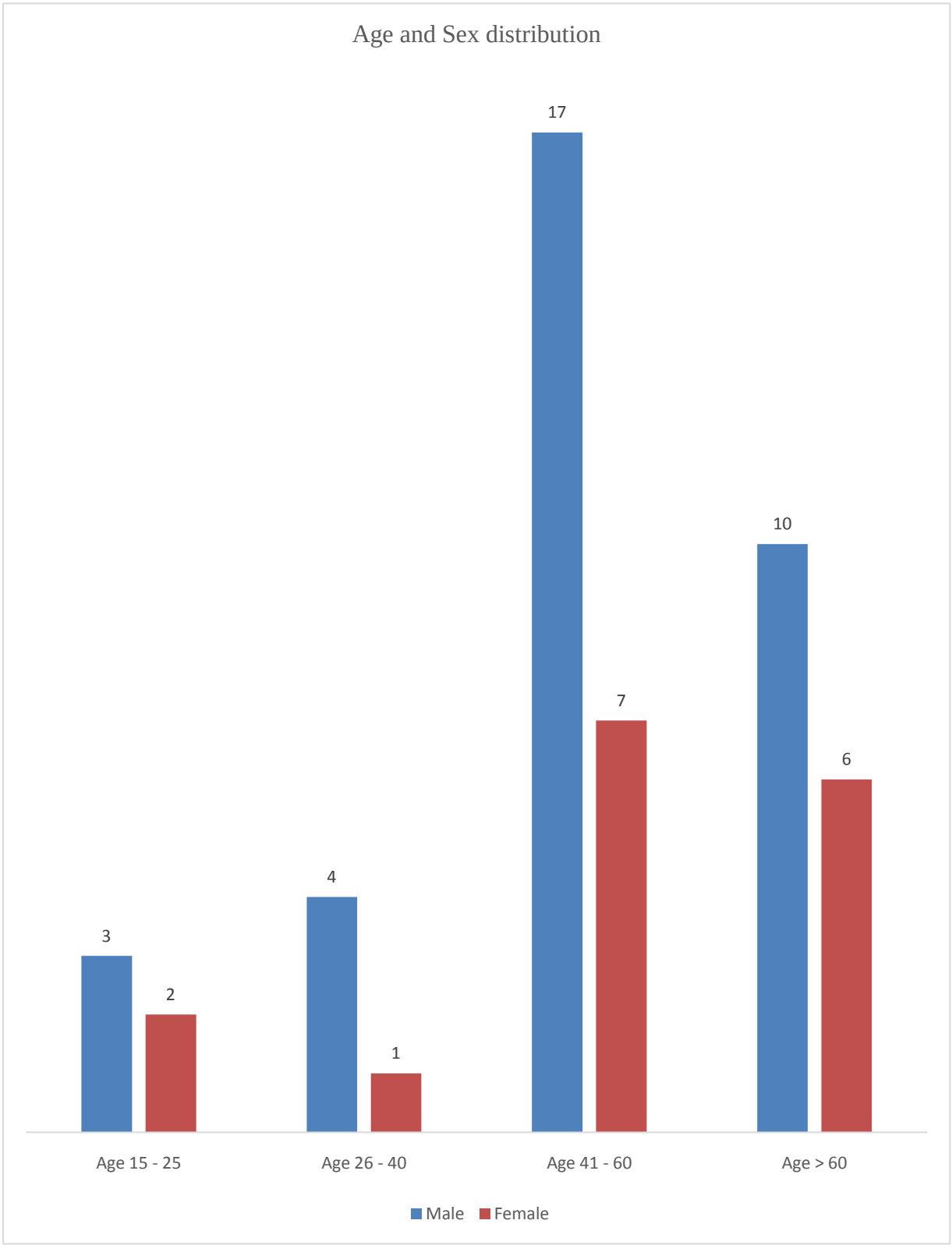


Figure 15: Age and sex distribution

In our population of patients, 58% (n=29) of the patients presented with fatigability, 16% (n=8) with chronic diarrhoea and 26% (n=13) with other symptoms like short pyrexia etc. No patient had bleeding tendencies or features of nervous system involvement. There were about 6 vegetarians with others consuming mixed diet. Five patients gave history of consuming alcohol.

Symptoms	n	Percentage
Fatigability	29	58%
Diarrhea	8	16%
Others	13	26%

Table 14: Presenting symptoms

On clinical examination, pallor was noticed in all patients in various degrees. Icterus was seen in 10 patients, hyperpigmentation of the kuckles in 7 patients. Organomegaly was noticed in 2 patients among them one had hepato-splenomegaly and one had splenomegaly.

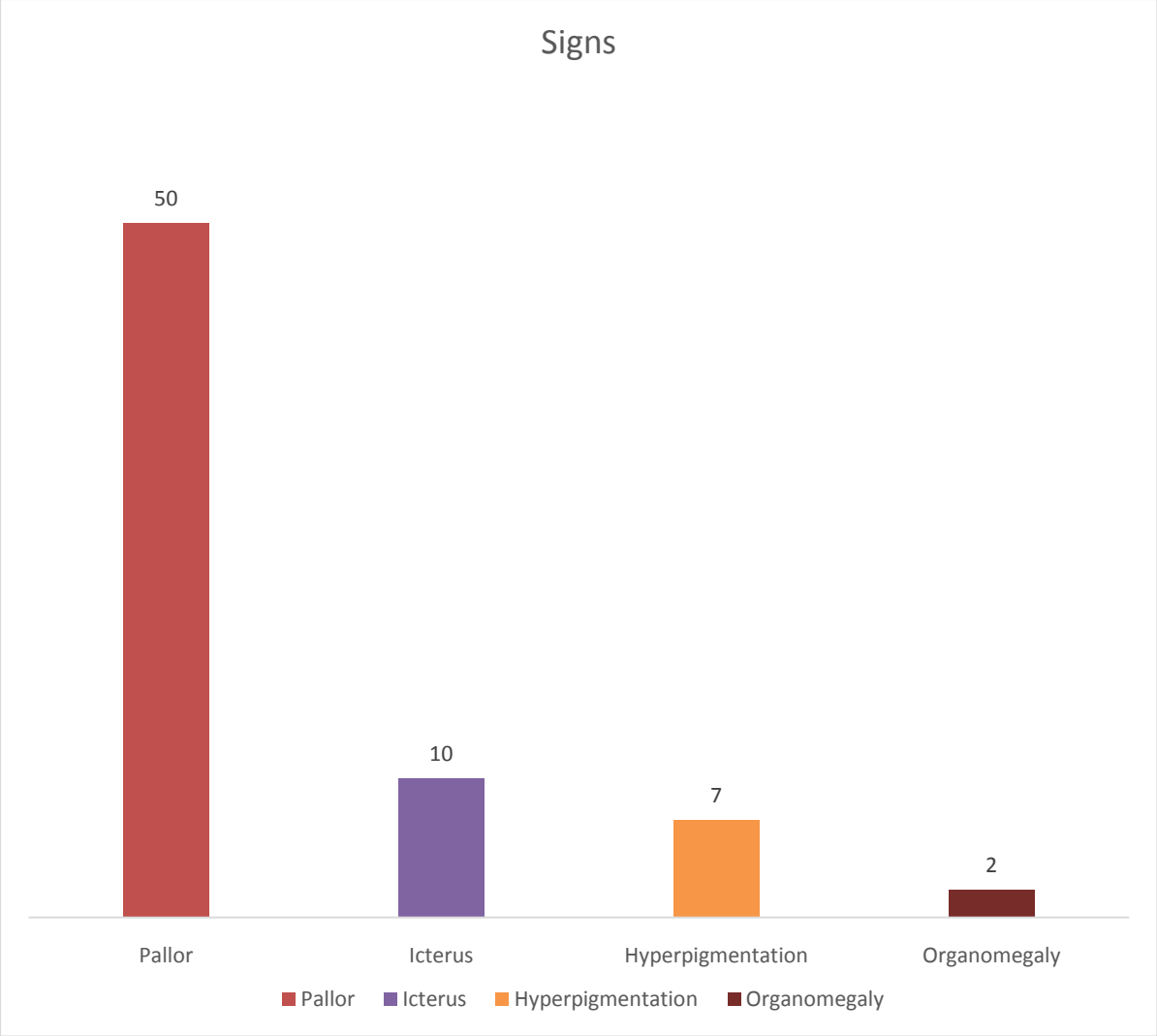


Figure 16 Clinical findings

In the study population 38 patients had low vitamin B12, 5 patients had low levels of both folate and vitamin B12 and 2 patients had low folate level alone. In the remaining five patients in whom both vitamin B12 and folate were normal, two had history of drug exposure.

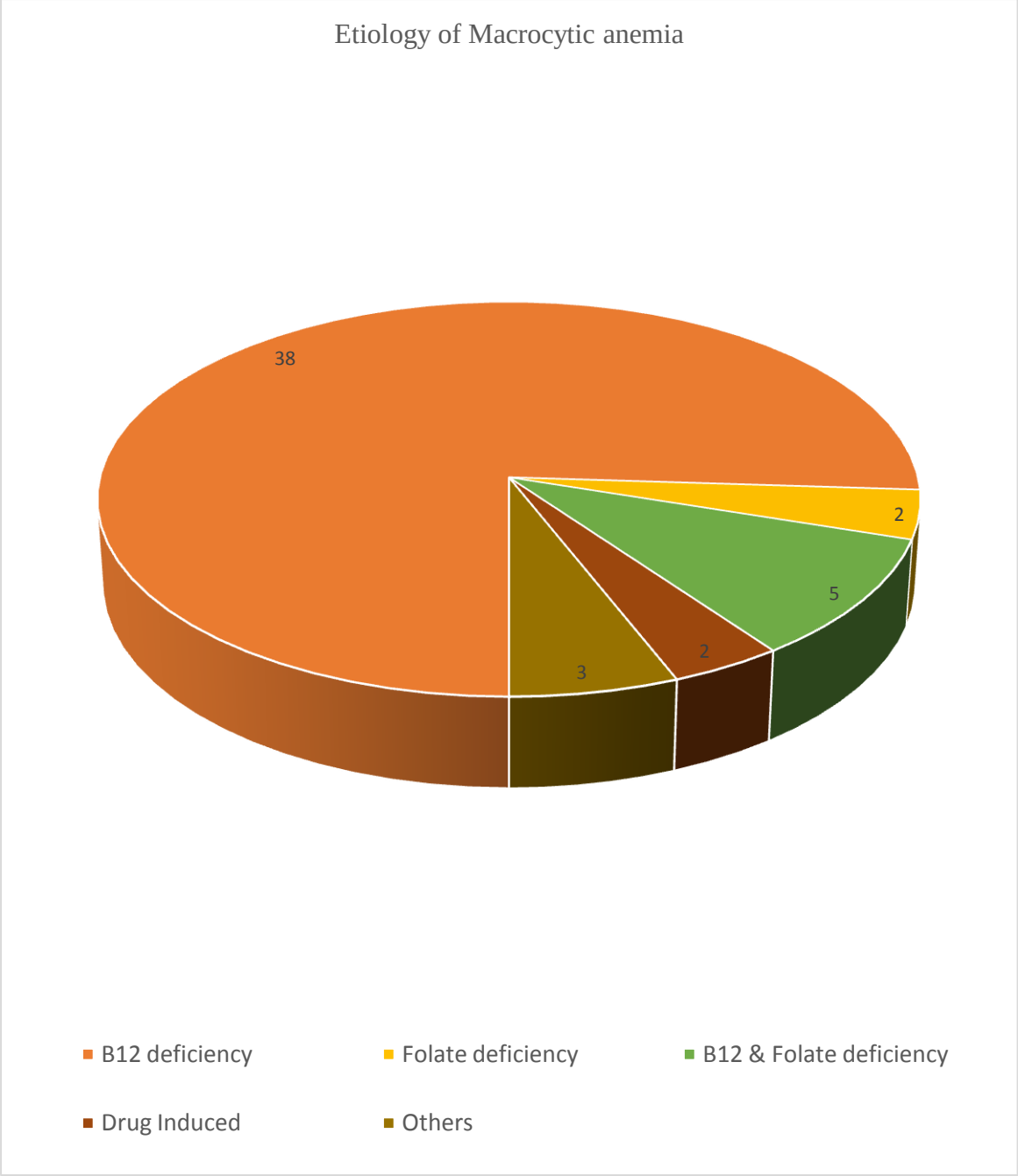


Figure 17: Etiology of macrocytic anemia

Among the remaining three, first patient a vegetarian presented with pancytopenia and macrocytosis. Bone marrow smear showed picture of megaloblastic anemia. Upper GI endoscopy showed scalloped duodenal folds, but duodenal biopsy showed non-specific inflammatory changes. Blood counts improved after treating with parentral vitamn B12.

Second patient a vegetarian presented with pancytopenia, macrocytosis, and hypersegmented neutrophils in peripheral smear. Bone marrow smear was suggestive of megaloblatic anema. Upper GI scopy showed scalloped duodenal folds and biopsy was suggestive of tropical sprue. Blood counts improved with supplementation of Vitamin B-12.

Third patient also a vegetarian presented with pancytopenia and macrocytosis in peripheral smear. Hemolytic work up was negative. Bone marrow smear was suggestive of megaloblastic anemia with ineffective erythropoiesis. Upper GI scopy showed scalloped duodenal folds, biopsy showed peptic duodenitis.

The hematological study at the starting point revealed 20 patients presented with anemia alone, 6 patients with bicytopenia (low haemoglobin and low platelets) and remaining 24 patients presented with pancytopenia.

The mean haemoglobin was 7.1, mean MCV was 115 and mean red cell distribution width (RDW) was 22.71 among the fifty patients.

Hematological Parameter	Mean ± SD
Hemoglobin	**7.1 ± 2.5**
MCV	**115.1 ± 10.3**
RDW	**22.71 ± 6.2**
Total Count	**4775 ± 2643**
Platelet Count	**123686 ± 100097**

Table 15: Hematological parameters

	Male	Female
Pancytopenia (n = 20)	**17**	**3**
Bicytopenia (n = 6)	**4**	**2**

Table 16: Sex distribution in patient presenting as pancytopenia and bicytopenia

The mean haemoglobin was 5.62 and mean platelet count was 49933 in the patients presented with bicytopenia. In the patients presented with pancytopenia the mean haemoglobin was 6, mean total white cell count was 2830 and mean platelet count was 51250.

Hematological Parameter in Bicytopenia	Mean ± SD
Hemoglobin	**5.62 ± 2.17**
Platelet Count	**49933 ± 26070**

Table 17: Hematological parameter in patients with bicytopenia

Hematological Parameter in Pancytopenia	Mean ± SD
Hemoglobin	**6 ± 1.99**
Total Count	**2830 ± 488**
Platelet Count	**51250 ± 32309**

Table 18: Hematological parameter in patients with pancytopenia

Hemoglobin	MCV	Male	Female
< 6	**113.56 ± 7.83**	**12**	**6**
> 6	**115.96 ± 11.39**	**22**	**10**

Table 19: Mean MCV and degree of anemia

When values of MCV were compared with the degree of anemia using Independent student T test it was found that there was no correlation (p=0.4) between the degree of anemia and the level of macrocytosis.

Vitamin B12 level		Mean MCV ± SD
< 100	n = 28	116.69 ± 10.89
101 – 190	n = 15	112.20 ± 6.83
> 190	n = 7	114.92 ± 12.50

Table 20: Level of vitamin B12(p/ml) and MCV

When the different ranges of levels of serum vitamin B12 were compared with mean of the mean corpuscular volume using Anova test, no correlation was found (p=>0.5).

Among 20 patients presented with pancytopenia, 16 had their serum vitamin B-12 lower than normal with mean 64.37. Among 6 patients presented with bicytopenia, all had their serum vitamin B-12 lower than normal with mean 76.83. Among 24 patients presented with anemia 21 had serum vitamin B-12 lower than normal with mean 98.24.

	Vitamin B-12 Mean ± SD
Pancytopenia	64.37 ± 31.41
Bicytopenia	76.83 ± 33.36
Anemia	98.24 ± 44.88

Table 21: Level of vitamin B12 (p/ml) in patients with pancytopenia, bicytopenia and anemia

When the various levels of low serum vitamin B12 were compared with presentation of patient with anemia, bicytopenia or pancytopenia using Anova test there was was no correlation (p=>0.5).

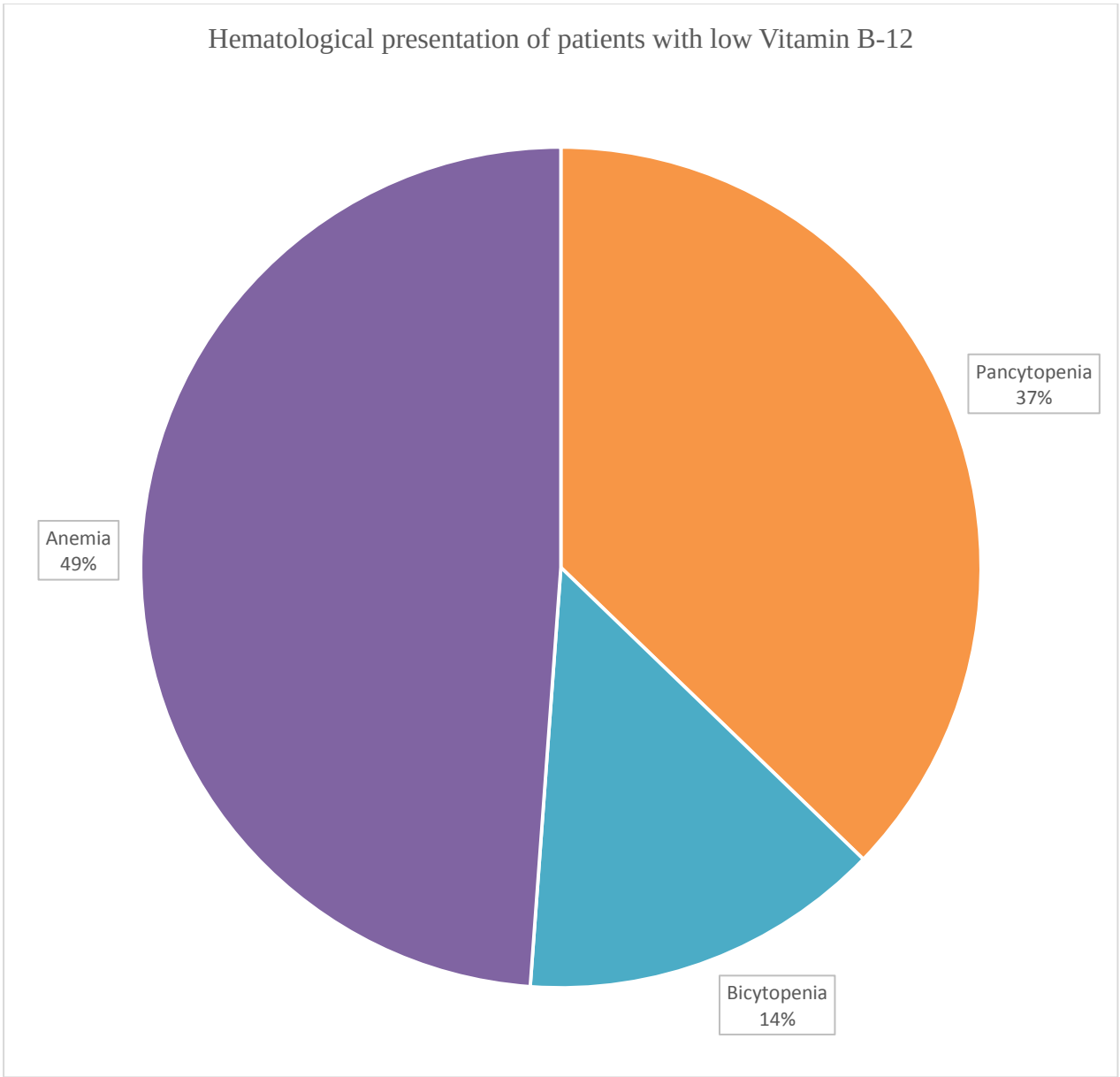


Figure 18: Hematological presentation of patients with low Vitamin B-12

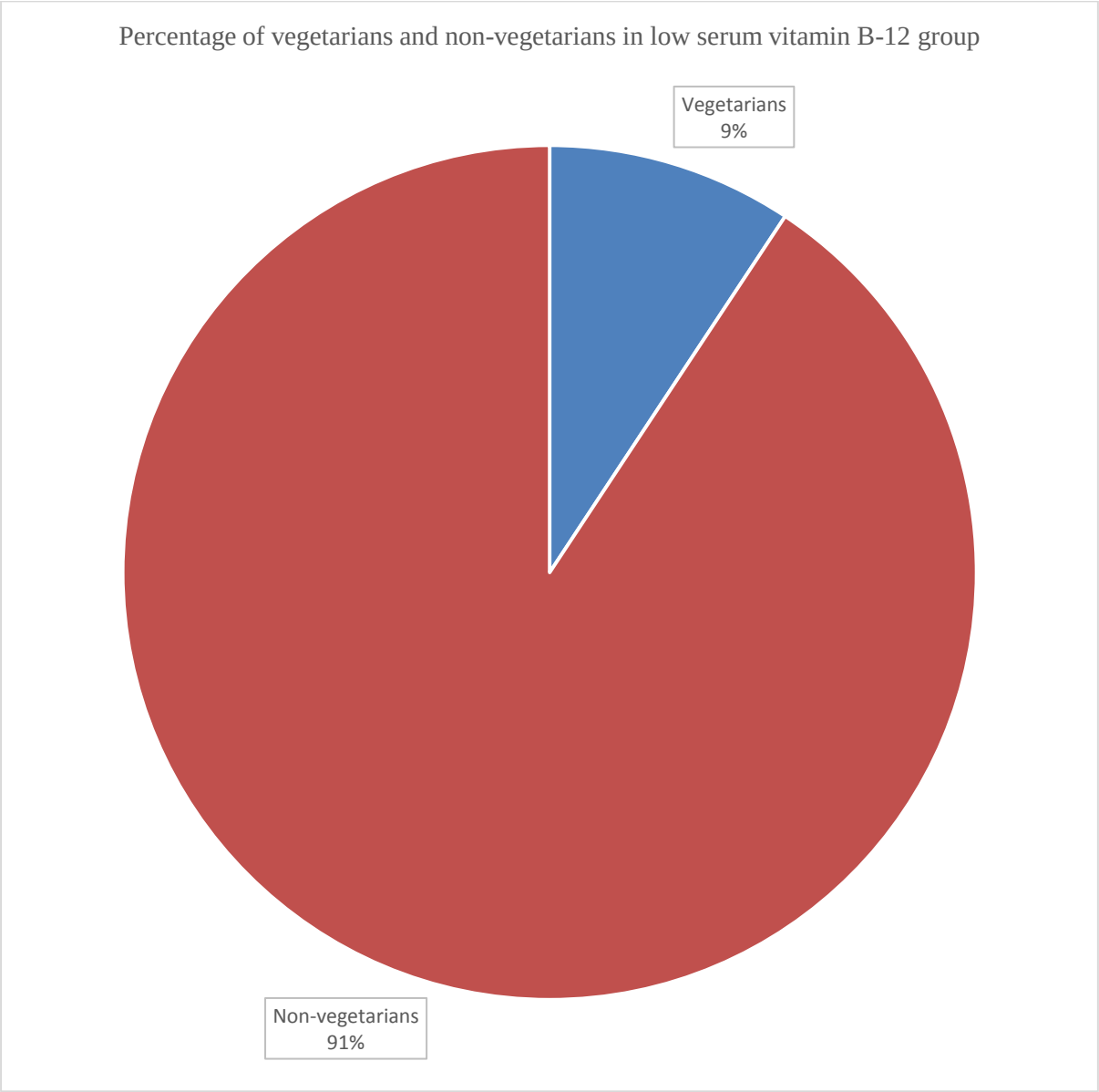


Figure 19: Percentage of vegetarians and non-vegetarians in low serum vitamin B-12 group

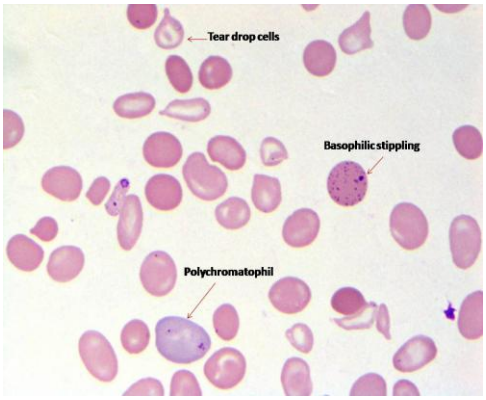


Figure 20 a

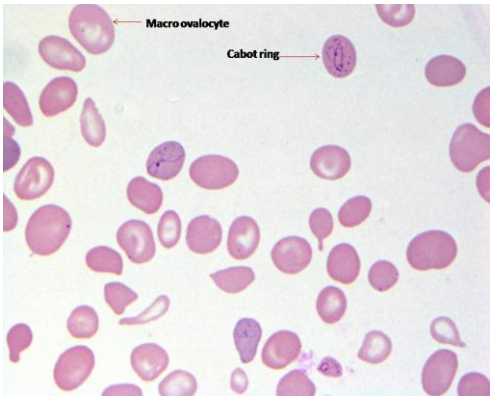


Figure 20b

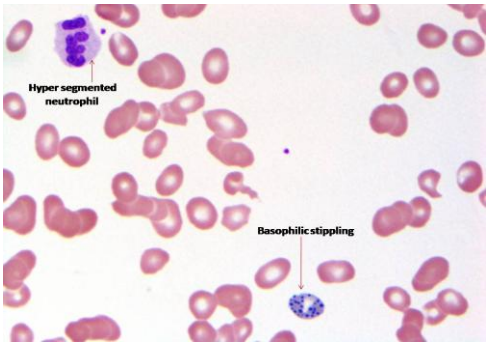
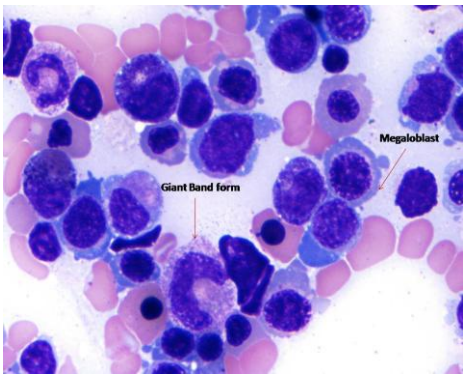


Figure 20c

Figure 20 a, 20b & 20c: peripheral smear features in our patients

On examining the blood peripheral smear hypersegmented nuetrophils or macroovalocytes was seen in 25 patients suggesting megaloblastic erythropoiesis.



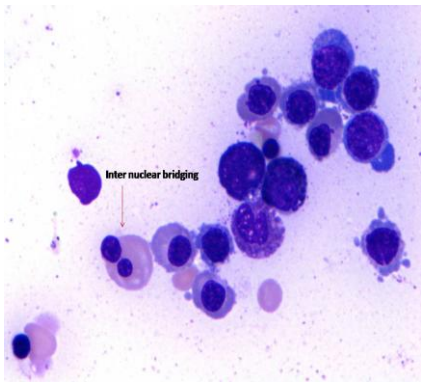


Figure 21a ***Figure 21b***

Figure 21a & 21b: Bone marrow aspiration smears in one of our patient

Among 50 patients, 29 patients who gave consent for bone marrow procedure underwent marrow aspiration and smear study and was found that all had megaloblastic erythropoiesis in their marrow.

Hypersegmented / Macro-ovulocytes	**Megaloblastic bone marrow**	
	Yes	**No**
Yes	25	0
No	4	0

Table 22: Megaloblastic features in peripheral smear and megaloblastic bone marrow

Different types of morphologically abnormal cell in the peripheral smear study include hypersegmented neutrophils, anisocytosis, macroovalocytosis, tear drop cells and nucleated red blood cells. These findings had strong association with megaloblastic erythropoesis of marrow.

In 5 patients who had not shown any features with hypersegmented neutrophils or macroovalocytes in the peripheral smear, 4 patients had low vitamin B12 and one had low folate levels.

Icterus was noticed as a principal finding in 10 patients. The mean of the total serum bilirubin in those patients was found to be 3.16 and all had increased indirect bilirubin.

Drug intake was seen in 3 of the patients. One was on sodium valproate for movement disorder and had associated low vitamin B12 and presented with pancytopenia. Among the other two in whom vitamin B 12 was low one was on phenytoin and sodium valproate for seizure and presented with pancytopenia. The other patient was on phenytoin for seizures and presented with anemia alone. All the three drug exposed patients had megaloblastic bone marrow.

Number of patients who had features suggestive of megaloblastic anemia in peripheral smears (macrocytosis, ovulomacrocytosis, aniso and poikilocytosis, hypersegmented neutrophils) was 25. All these patients had undergone bone marrow aspiration. Their bone marrow smears confirmed megaloblastic picture. Also four patients whom do not show typical megaloblastic peripheral smear picture showed megaloblastic bone marrow on aspiration smear.

Among the study people thirty four consented for upper GI scopy. Twenty six of them showed duodenal finding of either scalloped duodenal folds or folded duodenal margins. Other showed features of erosive gastritis, atrophic gastritis or antral gastritis. One had external gastric impression and the other had gastric polyp.

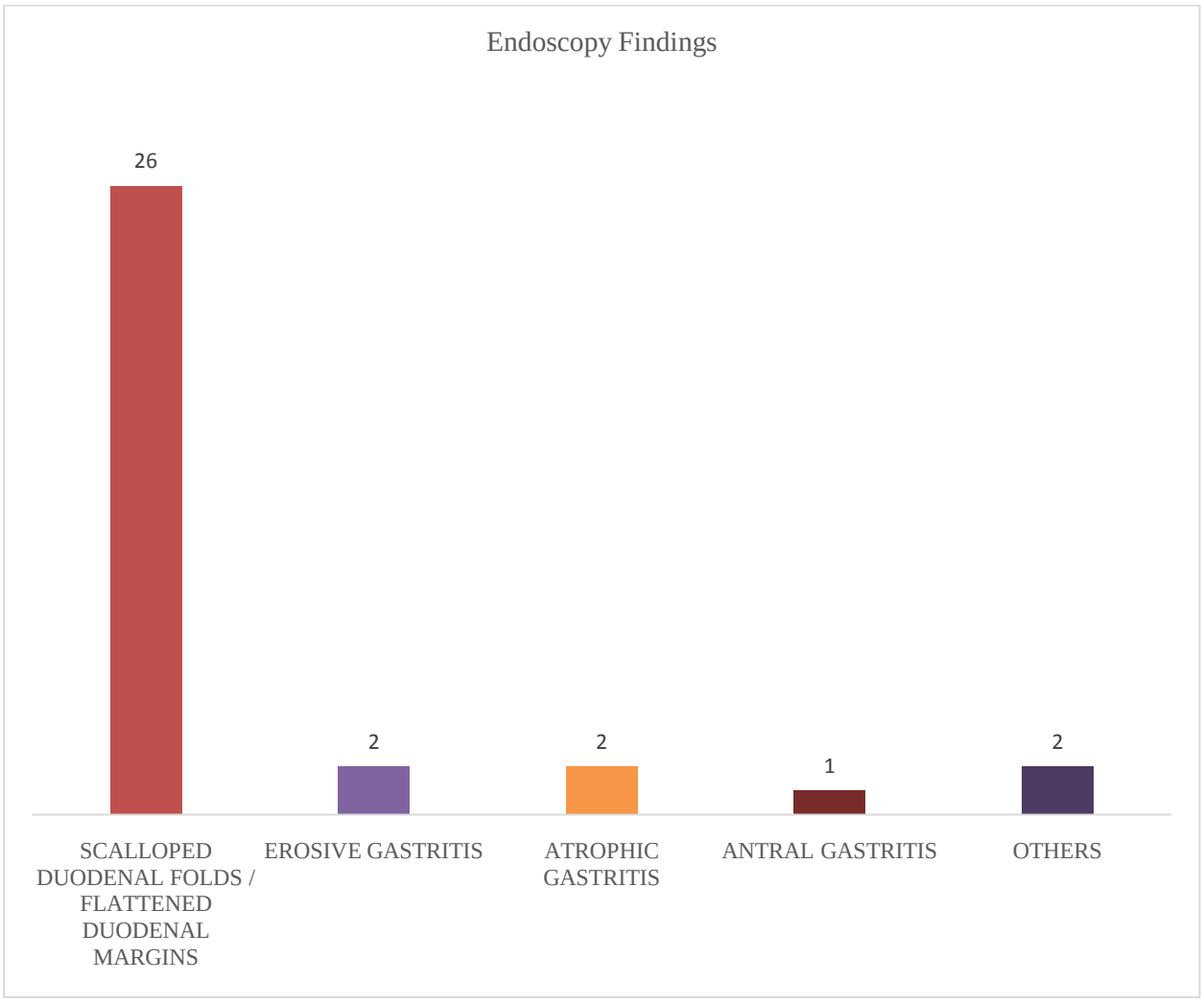


Figure 22: Endoscopy findings

Among twenty six patients in whom there were abnormal duodenal features fifteen showed features suggestive of tropical sprue in deep duodenal biopsy. Two patients who had normal duodenal findings had features of tropical sprue in biopsy.

Positive duodenal findings	**Tropical sprue**	
	Yes	**No**
Yes	15	11
No	2	5

Table 23: Positive duodenal endoscopic findings and tropical sprue

When positive finding in the duodenum during upper GI scopy and the diagnosis of tropical sprue with biopsy features were compared there was no statistical significance (p=0.2).

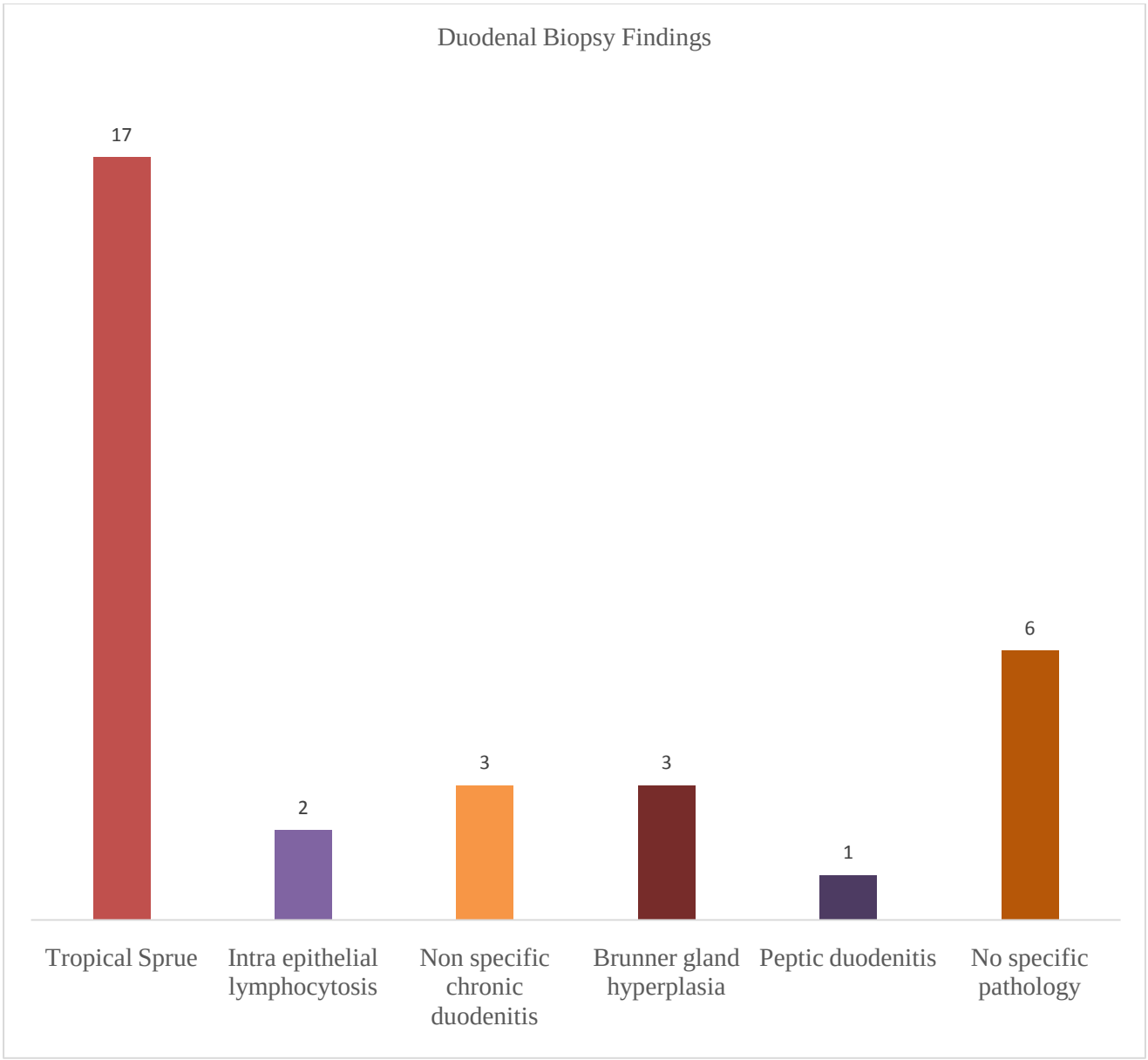


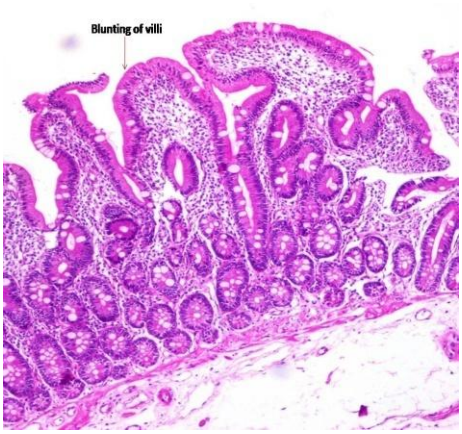
Figure 23: Duodenal biopsy findings

Among 7 patients presented with chronic diarrhoea 4 underwent UGI scopy and duodenal biopsy and all were found to have tropical sprue.

There were total of 13 males and 4 females with tropical sprue.

	Male	Female
Tropical Sprue	13	4

Table 24: Genderdistribution in tropical sprue



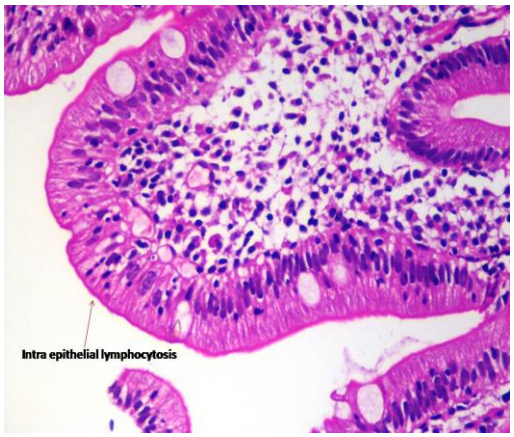


Figure 24a ***Figure 24b***

Figure 24a &24b: Findings in the biopsy from the duodenum in our patient

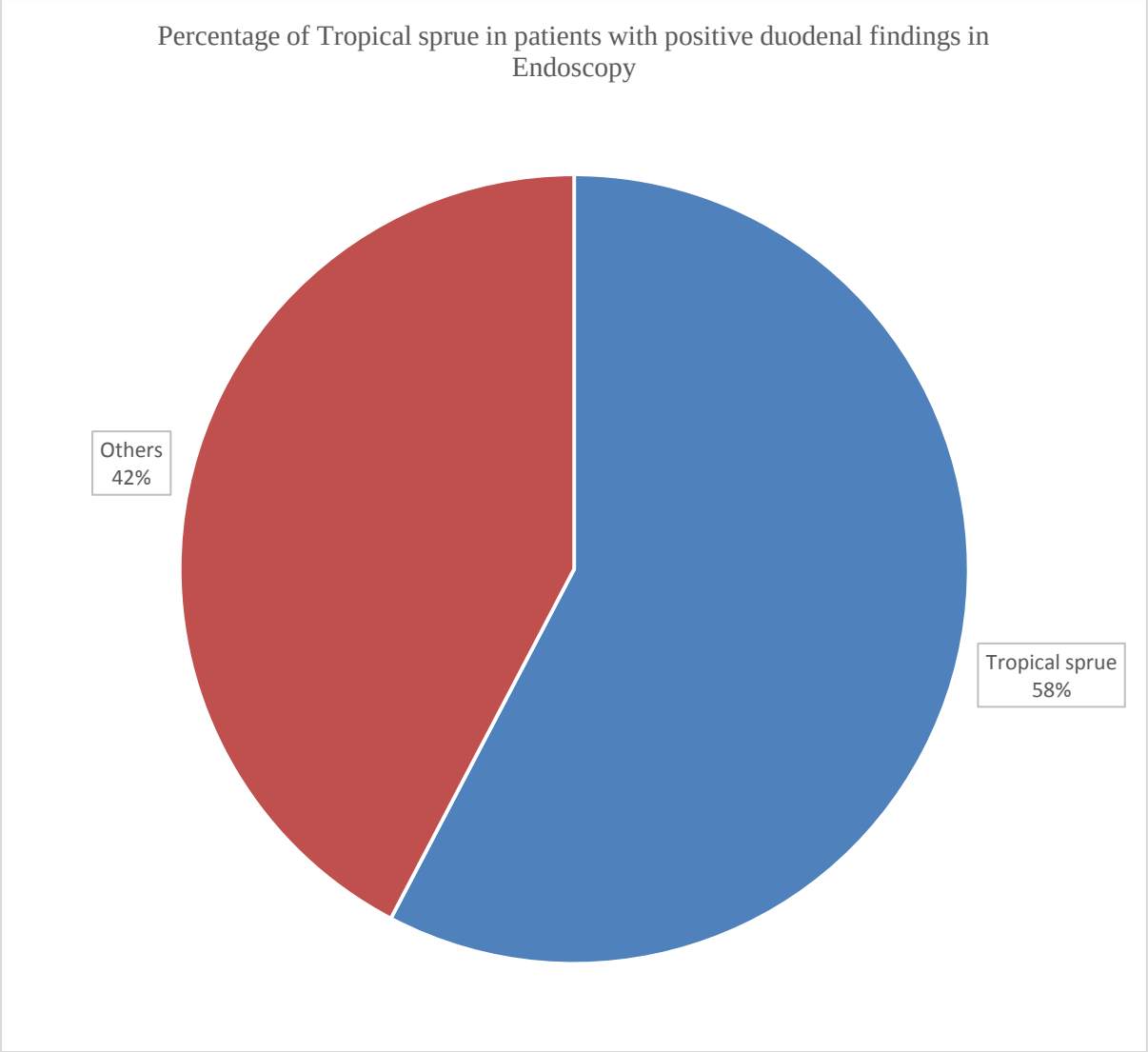


Figure 25: Percentage of Tropical sprue in patients with positive duodenal findings in Endoscopy

DISCUSSION

Macrocytic anemia has variable etiological factors.In our study population 76% had vitamin B12 deficiency,10% had deficiency both folate vitamin B12 and 4% each had folate deficiency and drug induced macrocytic anemia. McPhedran in his study on 100 consecutive patients, reported Low B 12 and low folate levels with megaloblastic erythroid cells was commonest reason which leads to macrocytic anemia[2] .This was consistent with our study.

But on the other hand in the study conducted by Colon-Otero excessive alcohol intake was commonest reason for macrocytosis followed by low vitamin B 12 and low folic acid levels [128]. In another study conducted in the year 2000 by Savage and co workers, reported, commonest reason for macrocytosis was drug exposure. Following it was the alcoholic liver disorders and reticulocytosis.

Study	Common cause identified
Our study	Megaloblastic
McPhedran	Megaloblastic
Colon-Otero	Alcohol abuse
Savage et al	Drug, Alcohol abuse

Table 25: Causes of Macrocytic anemia in various studies

We know there are variable clinical presentation of Vitamin B12 deficiency associated with megaloblastosis in the bone marrow, macrocytosis in the peripheral smear and a raised MCV. In our study population major presenting symptom was fatigability which was found in 58% of the patient and in about 16% of patient chronic diarrhoea was the presenting symptoms. No patient was presented with bleeding manifestation or had neurological symptoms.

In a study conducted in 100 patients by Tejas Shah and Tarun Rathod 96% presented with symptoms of fatigue, 30% and 42% of the study population had psychiatric and neurological symptoms, 2% had diarrhoea[77]. In another study by Salma Haq et al in a population of 80 patients 84% had fatigability, 34% had bleeding manifestation and no patients had neurological symptoms[12]. In a study conducted by Vineetha Unnikrishnan et al in a population of 60 patients 91.7% presented with fatigability, 36.7% had bleeding manifestation, 1.7% with diarrhoea and 10% with neurological symptoms.

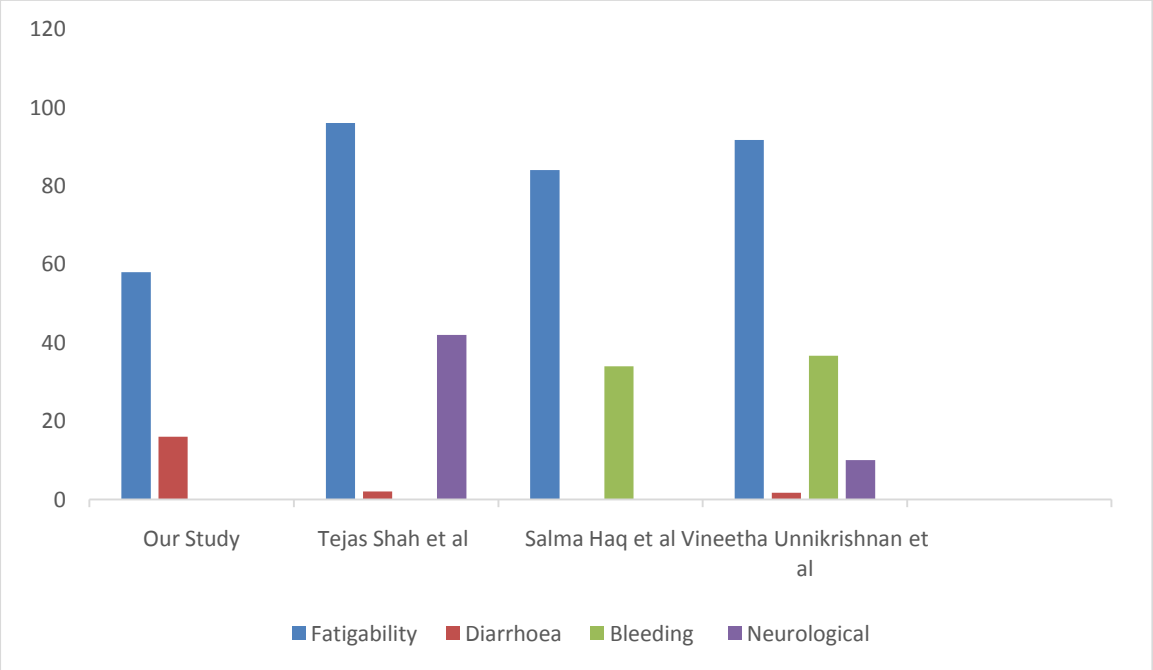


Figure 26 Comparison of presenting symptoms in various studies

In our patient population male and female percentage is 68 and 32 respectively. Male to female ratio was 2.1 : 1. About 80% of the patients were of the age above 40 years. Among them 48% were of the age between 41-60 years and 32% were above the age of 61 years.

In comparison, Salma haq et al study showed 17.6% of patients in age group 41-60 and 12.5% in age group above 60 years. In contrast to our study this study also included patients below the age 15 years.

In studies conducted in Caucesian population and patients in china, megaloblastic anaemia was accounted in old age group patients with equal sex distribution or predominanatly males.

But our study showed highest incidence among the patients between the age the age group of 10 and 30 years i.e (48% of patients) and there was a preponderance of women (71%).

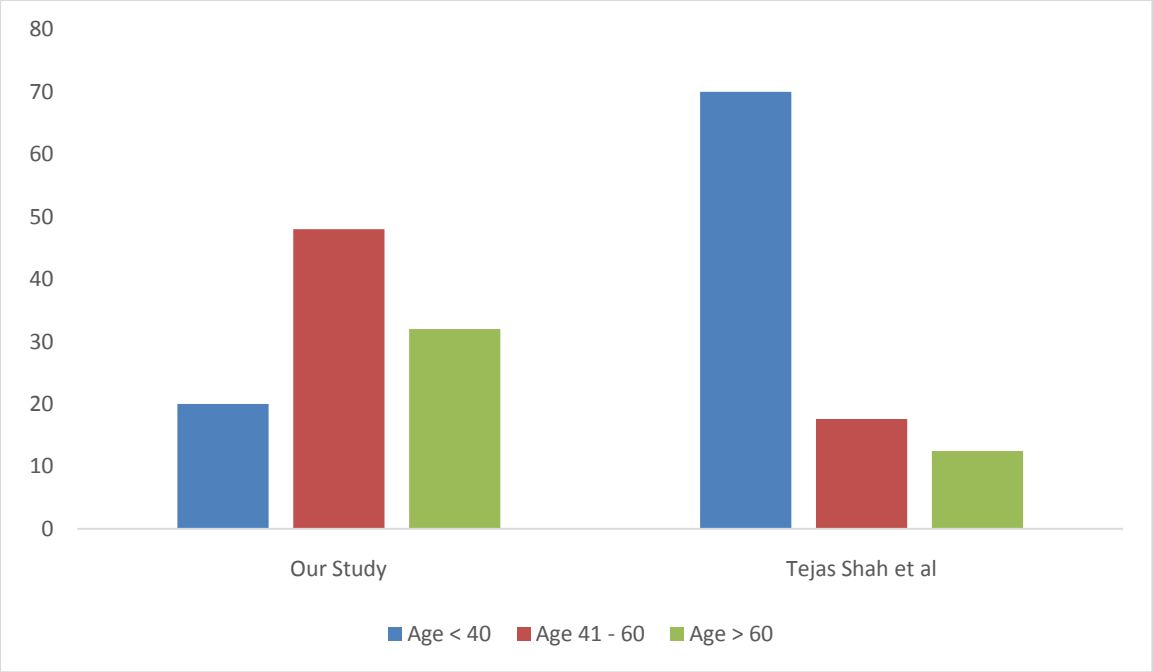


Figure 27 Comparison of age group with Tejas Shah et al

Comparing gender ratio with other studies, Vineetha Unnikrishnan et al had more Male : Female ratio as that of our study. But studies done by Tejas Shah et al and Uma Khanduri et al showed female predominance.

Study	Male : Female ratio
Our study	2.1 : 1
Tejas Shah et al	1 : 1.5
Uma Khanduri et al	1 : 1.4
Vineetha Unnikrishnan et al	1.8 : 11

Table 26: Gender distribution in various studies

In our study population clinical examination revealed pallor in 100%, icterus in 20%, skin hyperpigmentation in 7% and organomegaly in 4% of the population.

In the study by Tejas Shah et al 63% had pallor, 32% had skin hyperpigmentation and 12% had icterus.In the other study by Salma Haq et al 84% had pallor, 28% had jaundice and hepatomegaly and 48% percent had splenomegaly. In a study by Vineetha Unnikrishnan 35% had jaundice, 31.7% had splenomegaly, 28.3% had hepatomegaly and 23.3% had skin changes.

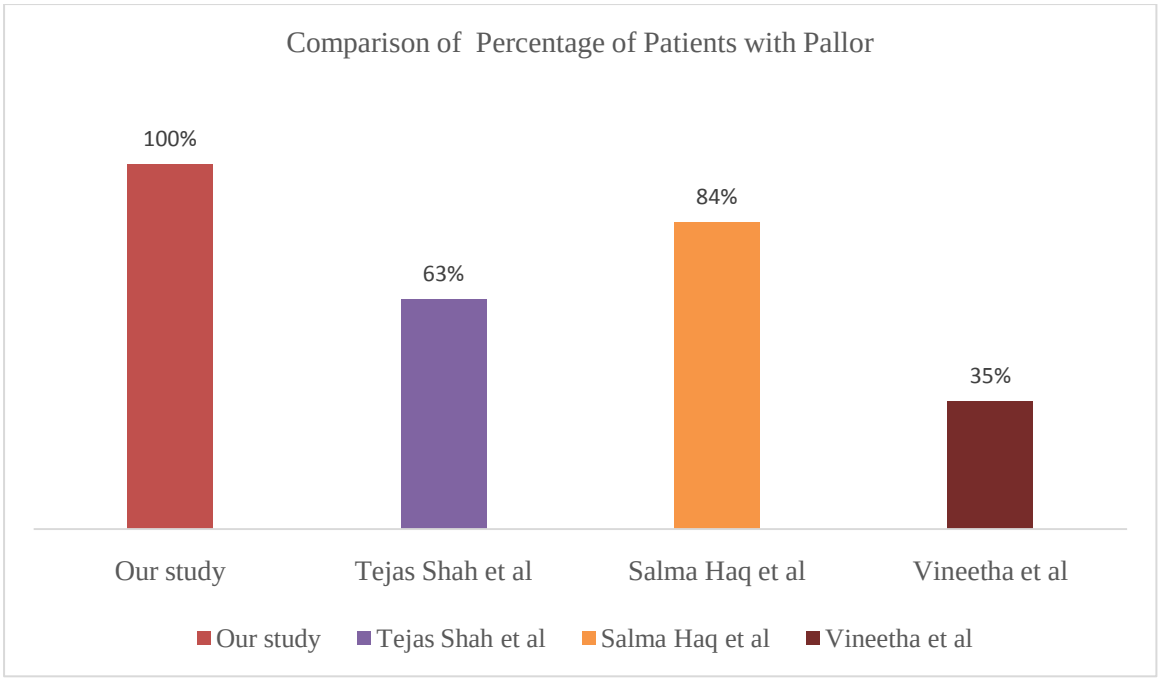


Figure 28: Comparison of presentation of patients with Pallor

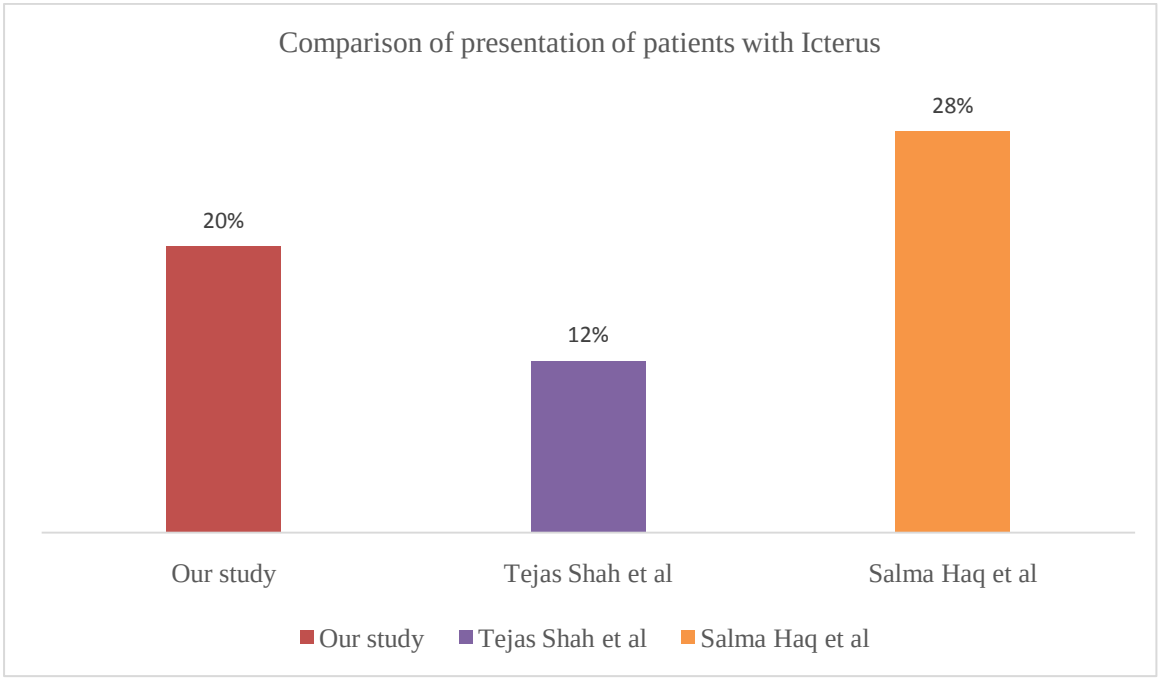


Figure 29: Comparison of presentation of patients with Icterus

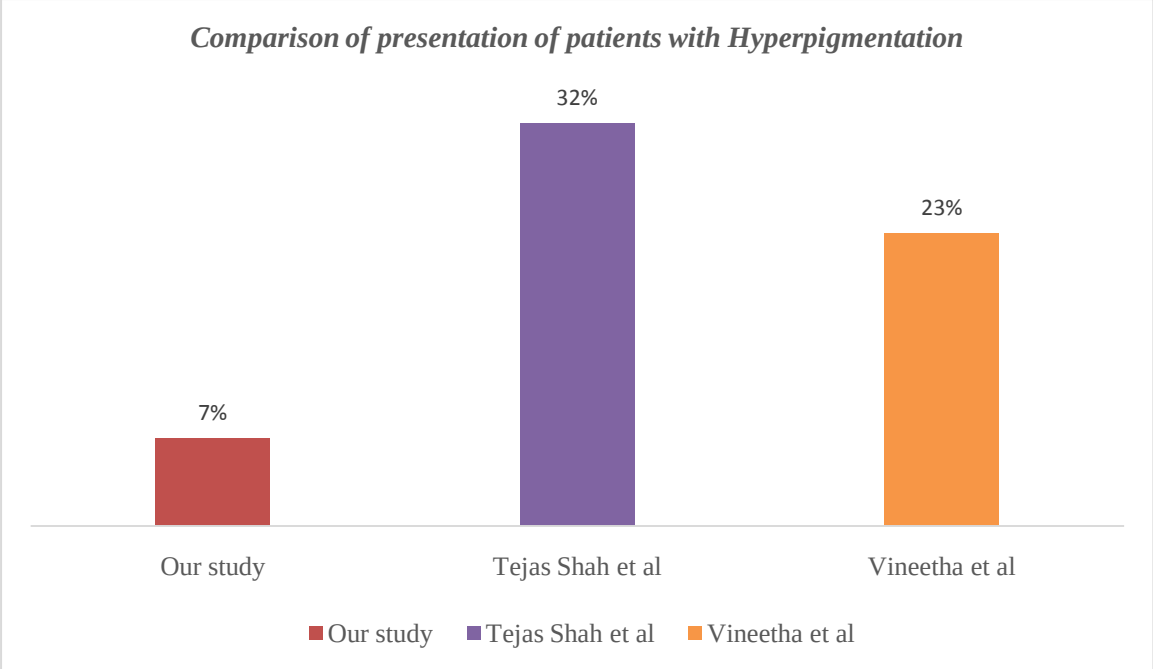


Figure 30: Comparison of presentation of patients with Hyperpigmentation

Thus when compared to the above studies our study population had lesser percentage of patients with jaundice, organomegaly and skin changes, and have more patients presenting with pallor.

On presentation in our study population 48% had pancytopenia, 20% had anemia and 12% had bicytopenia. In the study by Tejas Shah et al reported same percentage (48%) of patient with pancytopenia. In another study by Uma Kandhury et al 62% of pancytopenia.Salma haq et al study reported pancytopenia in 41% and bicytopenia in 60% .

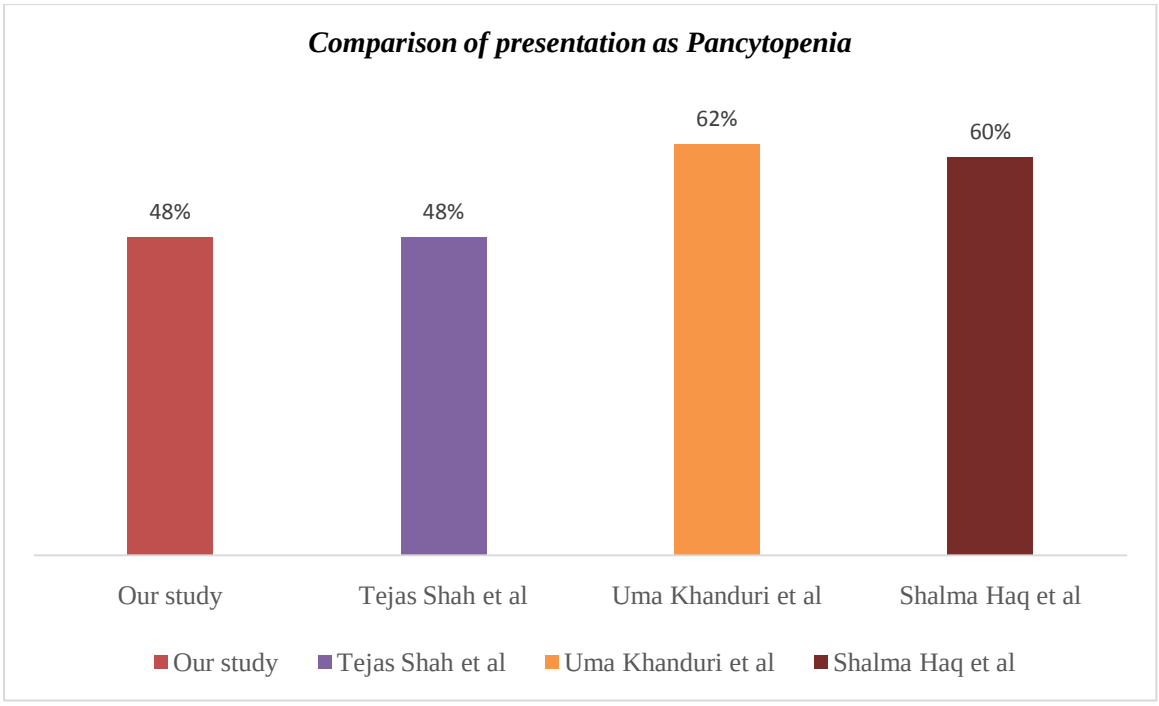


Figure 31: Comparison of presentation as Pancytopenia

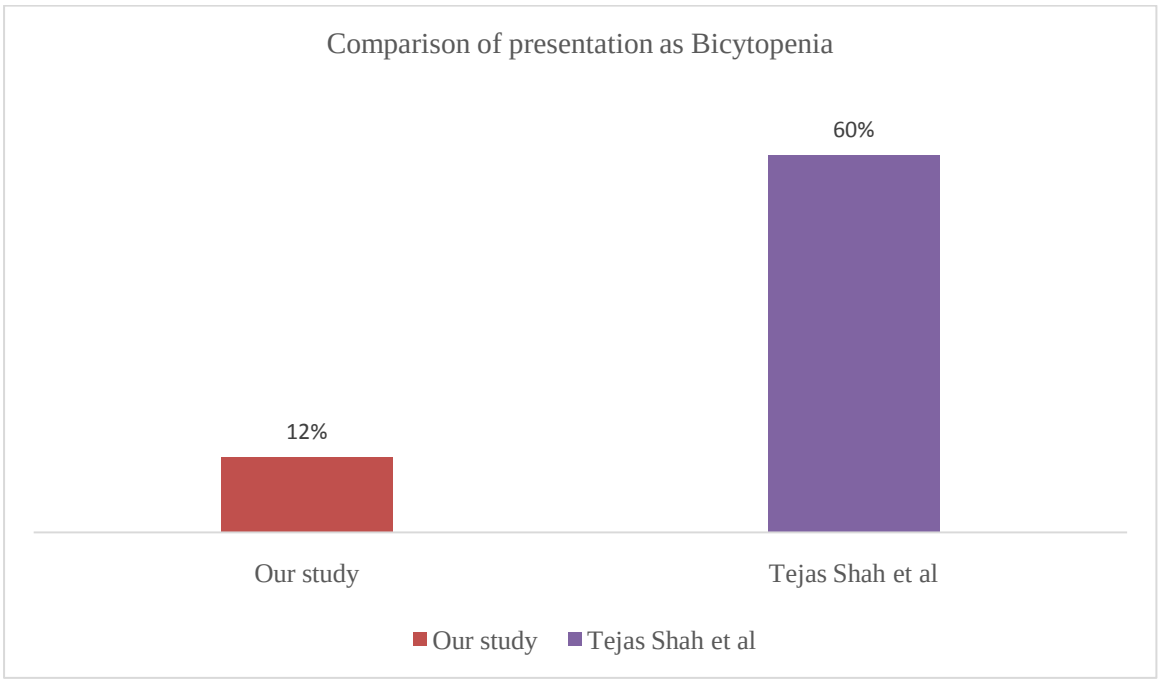


Figure 32: Comparison of presentation as Bicytopenia

Hematological Parameters	Our Study n=50	Vineetha Unnikrishnan n=60	Gulam Shah n=113
Hemoglobin	7.1 ± 2.5	5.6 ± 2.12	11.32 ± 2.15
Mean corpuscular volume	115.1 ± 10.3	106.5 ± 9.59	100.64 ± 19.77
Red cell distribution width	22.71 ± 6.2	20.7 ± 6.06	12.5 ± 2.5

Table 27: Comparison of hematologic parameters of the study population at presentation

Hematological Parameter	Our Study n=50	Vineetha Unnikrishnan n=60
Total WBC count	4775 ± 2643	1043 ± 2813
Platelet count	123686 ± 100097	109000 ± 110000

Table 28: Comparision of hematological parameters

In our study 36% patients presented with haemoglobin of less than 6 and 64% presented with haemoglobin more than 6. In comparison study by Vineetha unnikrishnan et al the percentage was 63.3% and 36.7% respectively.

Hemoglobin values	Mean corpuscular volume	
	Our study n = 50	Vineetha Unnikrishnan n = 60
< 6	113.56 ± 7.83	107.7 ± 8.64
	n = 18	n = 38
> 6	115.96 ± 11.39	104.5 ± 10.97
	n = 32	n = 22

Table 29: Comparison of haemoglobin and Mean corpuscular volume

In our study 91% of the population were on mixed diet and 9% were on vegetarian diet. In our study non vegetarians were commonly affected. In concordance with our study, other study conducted by Iqbal SP et al showed Vitamin B12 deficiency in 94% of non-vegetarians.

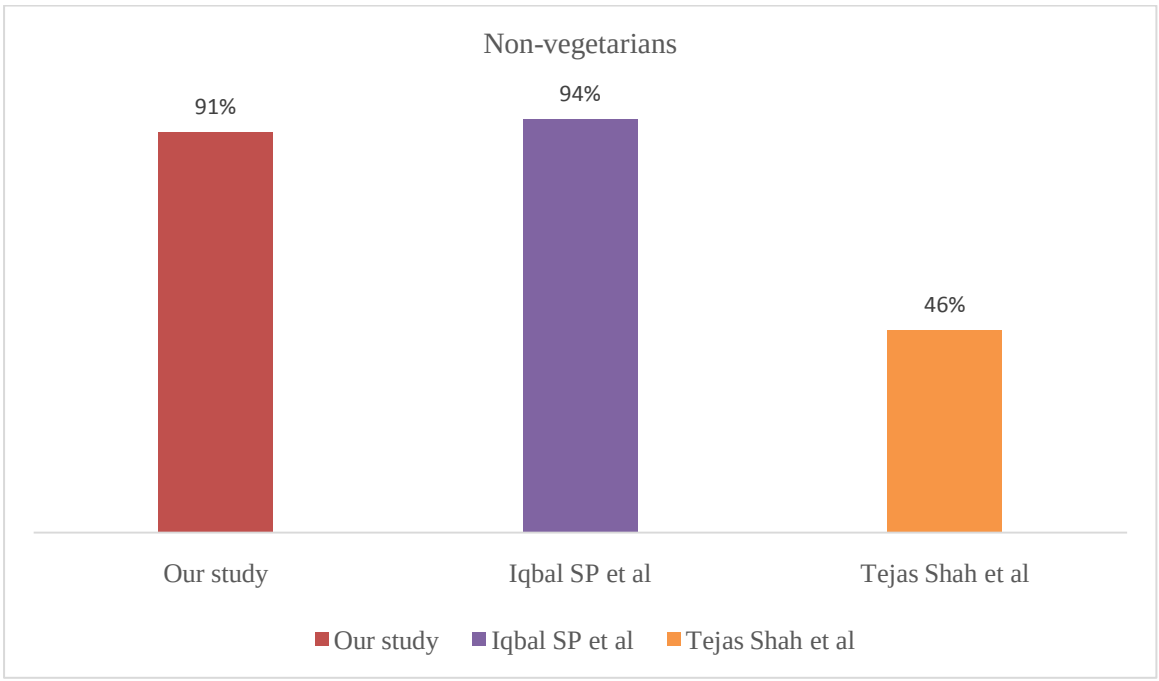


Figure 33: Non–vegetarians in various studies

But the data collected from Andrews L et al and SR Kankonkar et al it is known that vitamin B12 deficiency is higher in vegetarians compared to non-vegetarian.

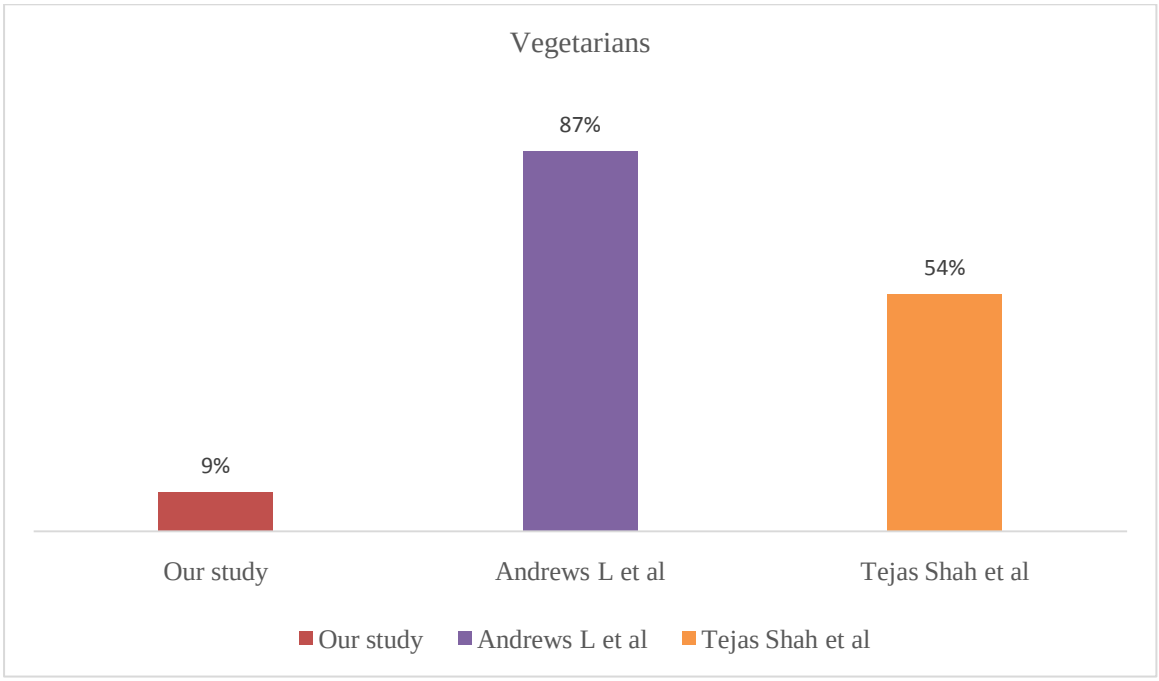


Figure 34: Vegetarians in various studies

In our study hypersegmented neutrophils were observed in about 50%. But Khanduri and Sharma's study showed hypersegmented neutrophils in all the patients. This study had same cut off value for MCV as our study.

In other two studies there were lower percentage of hyper segmented neutrophils than our study, 25.5% in the study by Punia Bhatia and 43% in the study by Vineetha unnikrishnan et al.

	Our study n=50	%	Vineetha et al n=60	%
Macrocytosis with other features of megaloblastosis	25	50	26	43
Macrocytosis without other features of megaloblastosis	25	50	34	57

Table 30: Comparison of peripheral smear findings

In our study population of 50 patients 66% (n=33) underwent Upper GI scopy. 52% of the total study population had tropical sprue. Among them 58% patient had positive duodenal finding in the upper GI scopy and 42% does not have any positive finding in the duodenum.

In comparison Pooja yadav et al showed Celiac disease as the commonest reason for (65%) mal-absorption following which was tropical sprue (22%). The same study demonstrated that most patients with tropical sprue had normal duodenal Folds (85.7%) while, the appearance of duodenal folds was abnormal in 82% patients with celiac Disease.

In another study conducted in north india by Ranjan P et al showed 39.3% of tropical sprue in their study population.

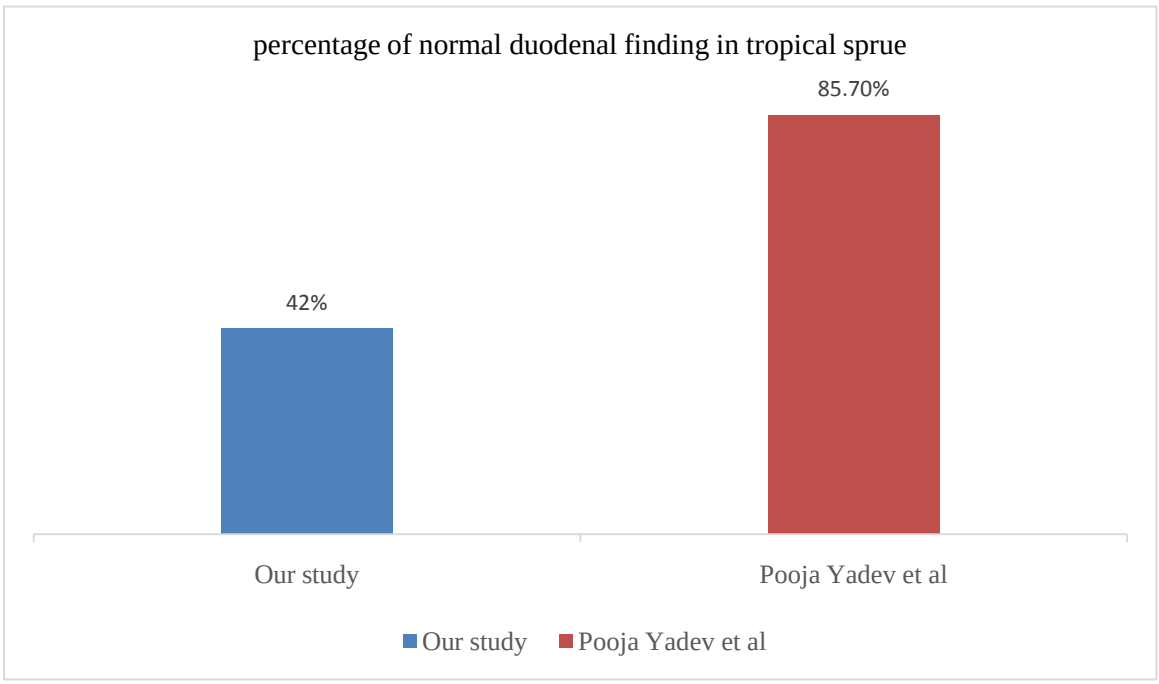


Figure 35: Comparison of percentage of normal duodenal finding in tropical sprue

LIMITATIONS OF THE STUDY

The following are the limitations of the study

1. The study was done in a tertiary care hospital and only patients with macrocytic anemia were included. Henceforth, quite a few number of patients with vitamin B12 and folate deficiency whom did not have anemia might have been missed. So the real prevalence of B12 deficiency could not be identified from this study.

2. In patients presented with nutritional deficiency complete work up of malabsorption like stool examination for ova and cyst, quantitative assay of stool fat, d-xylulose test, upper gastrointestinal endoscopy and biopsy was not done in all.

CONCLUSIONS AND RECOMMENDATIONS

- Even though many diseases might lead to macrocytic anemia our study shows megaloblastic macrocytosis as the most common cause.
- This demonstrates that megaloblastosis still remains the most significant reason for macrocytic anemia in our population which causes substantial morbidity. Low serum B12 vitamin was leading cause of megaloblastic anemia.
- Considering megaloblastic anaemia as one of the differentials in pancytopenia is important.
- Bleeding was not a presenting complaint in patients with thrombocytopenia due to B12 deficiency.
- We observed in our study that being a non vegetarian does not protect against Vitamin B12 deficiency.
- Although our study had very small number of cases with drug exposure, clinical history of drug intake is essential in evaluating for megaloblastic anemia.
- Macro-ovalocytes and hyper-segmented neutrophils occurring in peripheral blood smear would significantly favour towards diagnosing megaloblastic anemia.
- The severity of anemia did not have any correlation with the levels of serum B12 or the degree of macrocytosis.
- Upper GI endoscopy with deep duodenal biopsy should be done in all patients with megaloblastic anaemia.

- The prevalence of tropical sprue is also quite great. The high prevalence of tropical sprue as established in this study has not been revealed in any other study before. This emphasises the significance of evaluating tropical sprue in a suitable settings to the clinician.

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APPENDIX A

PROFORMA

Patient ID :

Age : Sex: Occupation:

Ip number :

Date of admission:

Presenting complaints:

History : Thyroid disease/Drug intake/Alcohol consumption/vegetarian or non-vegetarian

Clincal findings: Pallor/Icterus/skin hyperpigmentation/Organomegaly

Investigations:

Total count …… Hemoglobin ……. Platelet count ……

MCV …… RDW ……..

Peripheral smear ……..

Reticulocyte count ……..

Serum bilirubin …….. Direct bilirubin …… LDH …….

Vitamin B12 …… Folic acid …….

Bone marrow aspiration ……..

Bone marrow biopsy ……

UGI scopy ……

Duodenal biopsy ……..

Master Chart 1

S.No	Age	Sex	Hemoglobin	MCV	Total Count	Platelet	RDW	Vitamin B12	Folic Acid	TSH	Serum Bilirubin	Fatigability	Diarhoea	Drug Exposure	Veg/ NVeg	Anemia	Icterus	Hyperpigmentation	Organomegali	Peripheral Smear
1	70	F	5.4	123.4	4500	294000	24.5	138	16.6	7.39	N	Yes	NO	No	NV	Yes	NO	Yes	NO	Dimophic,Predominantly Macrocytic, Tear Drop,Elliptocytes,Sperocytes.
2	36	M	8.9	112	3100	80000	15.2	30	12.3	1.81	N	Yes	NO	No	NV/ALC	Yes	NO	Yes	NO	Pancytopenia, Macrocytosis
3	35	M	6.1	113	3300	8100	21.2	30	15.04	#	Y	Yes	NO	No	NV	Yes	Yes	NO	NO	Macrocytic,Mild Leukopenia,Thrombocytopenia,Elliptocytes,Polychromotophils
4	65	F	6.7	137.5	9600	12700	23.7	40	#	0.85	N		Yes	No	NV	Yes	NO	NO	NO	Macrocytic
5	74	F	10.8	98.8	7900	279000	14.8	181	4.98	4.99	N	Yes	NO	No	NV	Yes	NO	NO	NO	Macro
6	45	F	7.5	107.8	3500	209000	21.1	135	11.92	3.96	N	Yes	NO	No	NV	Yes	NO	NO	SPLEEN	Macro,Oval Macro,Tear Drop.Nrbc
7	55	F	7.3	105.2	1660	140000	26.9	137	0.94	2.87	N	NO	Yes	No	NV	Yes	NO	NO	NO	Macro And Micro,Hypersegmented, Neutrophilsoval Macro, Tear Drop,Poly Chromoto
8	62	M	7.2	110.9	7100	268000	20.8	76	2.79	0.05	N	NO	NO	No	V	Yes	NO	NO	NO	Macro
9	57	F	3.7	120.5	1400	48000	37.3	30	10.7	4.26	Y	Yes	NO	No	NV	Yes	Yes	Yes	NO	Pancyto,Macro,Hypersegmented Neutrophils, Oval Macro, Tear Drop.Nrbc
10	73	F	5.1	110.8	3200	154000	27.1	33	6.57	26.2	Y	NO	NO	No	NV	Yes	NO	NO	NO	Macro,Tear Drop,Poly Chromo,
11	55	M	5.8	110	3300	47000	17.5	55	#	#	N	Yes	Yes	No	NV/ALC	Yes	NO	Yes	NO	Macranisopoi,Polychromo,Nrbco
12	18	M	7.1	127	3400	60000	22.2	997	9.28	#	Y	NO	NO	No	V	Yes	NO	NO	NO	Pan,Macro,Tear Drop,Poly Chromot,Nrbc
13	57	F	10	109.4	8000	324000	18.7	122	5.59	6.52	Y	Yes	NO	No	NV	Yes	NO	NO	NO	Macro
14	71	M	11.1	113.8	1110	157000	20.7	62	11.29	1.09	N	NO	NO	No	NV	Yes	NO	NO	NO	Macro,Target,Sperocytes
15	48	M	9.9	101	3600	143000		820	1.5	1.57	N	NO	NO	No	NV	Yes	NO	NO	NO	Macro,
16	25	F	6.1	119.5	12600	202000	16.7	79	7.14	4.49	N	NO	NO	No	NV	Yes	NO	NO	NO	Macro,MACRO OVULOCYTES,HYPER SEG NEUT,
17	17	F	2.1	95.7	2700	15000	37	235	3.36	#	N	NO	NO	Yes	NV	Yes	NO	NO	NO	Aniso,Poi,Tear Drop
18	75	F	8.2	98.3	8100	37600	23.2	30	11.48	#	N	Yes	NO	No	V	Yes	NO	NO	NO	Macro
19	45	M	7.1	113.3	2900	108000	18.7	50	20	3.03	Y	Yes	NO	No	V	Yes	NO	NO	NO	Ovuloctes,Polychro,Tear Drop,Target
20	87	M	8.3	127.3	11700	320000	18.2	576	2.31	#	N	NO	NO	No	NV	Yes	NO	NO	NO	Macro,Nrbc,Hyperseg Neutr
21	54	M	12.9	104.3	6200	127000	16.5	132	9.38	#	Y	NO	NO	No	NV/ALC	Yes	Yes	NO	NO	Poly Chr,Nrbc
22	46	M	4.3	112.4	2600	29000	19.4	110	3.03	3.13	Y	NO	NO	No	NV/ALC	Yes	Yes	NO	LIVER/SPLEEN	Macro,Oval Macro,Tear Drop,Nrbc,Hyperseg Neutro
23	30	M	8.1	133.4	6500	212000	17.4	30	11.62	75.8	N	Yes	NO	No	NV	Yes	NO	Yes	NO	Macro,Ovalmacro,Polychrom,Hyperseg
24	48	F	10.9	112.7	10800	212000	16.4	120	3.2	6.8	N	NO	Yes	No	NV	Yes	NO	NO	NO	Macro,Poly,Hyperseg
25	54	M	6.6	114	4500	30000	24.9	113	6.93	1.75	N	NO	NO	No	V	Yes	NO	NO	NO	Macro,Ovalmacro,Polychrom,Hyperseg
26	65	M	6.8	124.6	3900	48000	30.6	99	2.81	#	N	Yes	NO	No	NV	Yes	NO	NO	NO	Pan,Tear,Polychrom,Hyperseb,Nrbc
27	55	M	8.5	110.5	5100	143000	26.5	161	14.68	1.38	N	Yes	NO	No	NV	Yes	NO	NO	NO	Macro,Macro Ovulocytes,Hyperseg Neut,
28	58	M	4	124	2900	10000	21.8	84	4.3	2.44	N	Yes	Yes	No	NV	Yes	NO	NO	NO	Pan,Macro,Nrbc

#-not done

Master chart 1 continued

29	70	M	5.2	112.9	2700	52000	25	67	15.75	0.59	Y	Yes	NO	No	NV	Yes	Yes	NO	NO	Pan,Oval Macr,Polychrom,Hyperseg Neut
30	45	M	5.8	109.9	2400	94000	17	224	5.39	2.23	N	Yes	NO	No	V	Yes	NO	NO	NO	Pan,Ova,Tear,Polychro
31	60	M	7.2	119.1	4100	154000	23.9	125	#	1.75	N	Yes	NO	No	NV	Yes	NO	NO	NO	Dim,Predom Macro
32	70	M	7.7	111.9	5400	51000	23.6	90	5.27	1.38	N	NO	NO	No	NV	Yes	NO	NO	NO	Bicyt,
33	24	M	11.3	124.8	5300	152000	18.4	111	#	1.6	N	NO	Yes	No	NV	Yes	NO	NO	NO	Macro,Hyperseg Neut
34	50	F	8.1	143.2	7900	366000	19.3	93	#	8.45	N	NO	Yes	No	NV	Yes	NO	Yes	NO	Macro,Hyperseg Neut
35	65	F	11.6	116.8	6700	277000	13	140	#	3.02	N	NO	NO	No	NV	Yes	NO	NO	NO	Macrocytosis,
36	70	M	4.6	114.3	2500	6400	20	936	#	#	Y	Yes	NO	No	NV	Yes	Yes	Yes	NO	Pan,Oval Macr,Polychrom,Hyperseg Neut
37	72	M	10.5	115.4	2800	9500	16.4	142	1.62	#	N	NO	NO	No	NV	Yes	NO	NO	NO	Pancytopenia,
38	36	F	2.2	115.7	6800	21000	38	91	>20	#	N	Yes	NO	No	NV	Yes	NO	NO	NO	Bicyto,Oval Macro,Poly Chr,Hyperseg Neut
39	58	F	4.6	115.3	2400	70000	20.4	30	10.85	#	Y	Yes	NO	No	NV	Yes	Yes	NO	NO	Pan,Polychr,Tear,Ellipto
40	34	M	10.6	95.1	6400	344000	20.4	63	7.37	#	Y	Yes	NO	No	NV/ALC	Yes	NO	NO	NO	Macro,Oval Macro,Poly,Target,Elipto,Nrbc
41	48	M	5.9	110.8	3000	47000	38.8	50	#	#	N	Yes	NO	Yes	NV	Yes	NO	Yes	NO	Pancytopenia
42	51	M	8.9	109.7	2500	120000	19.5	60	#	1.65	N	NO	Yes	No	NV	Yes	NO	NO	NO	Pancytopenia
43	40	M	3.5	117.1	4600	59000	20.8	32	#	2.24	Y	Yes	NO	No	NV	Yes	NO	NO	NO	Macro,Poly,Hyperseg,Nrbc,Ellipto
44	20	M	4.7	129.3	7200	172000	23.4	219	10.63	#	Y	Yes	NO	Yes	NV	Yes	Yes	NO	NO	Macro,Tear Drop,Poly Chromo,Nrbc,
45	58	M	6.4	132.6	4300	151000	25.4	50	>20	2.58	Y	Yes	NO	No	NV	Yes	Yes	NO	NO	Macro,Macro Ovulocytes,Hyperseg Neut,Nrbc,
46	51	M	5	99.7	2800	43000	29.2	55	4.53	6.64	Y	Yes	NO	No	NV	Yes	NO	NO	NO	Pan,Mac,Macro Ovulo,Ellipto
47	63	M	4.6	113.9	3200	43000	20	88	6.85	4.68	N	Yes	NO	No	NV	Yes	NO	NO	NO	Pan,Mac,Macro Ovulo,Ellipto,Hyperseg, Polychrom
48	60	M	7.2	121	3000	72000	23	52	9.87	#	N	Yes	NO	No	NV	Yes	NO	NO	NO	Tear,Pan,Tar
49	59	M	5.5	108.5	4500	101000	36.3	105	13.25	1.7	N	Yes	NO	No	NV	Yes	NO	NO	NO	Macro,Oval Macro,Polychrom,
50	43	M	7.6	127	3100	62000	20.1	53	9.7	1.24	Y	Yes	NO	No	NV	Yes	Yes	NO	NO	Pan,Mac,Macro Ovulocy,Hyperseg

#-Not done

Master Chart 2

S.NO	AGE	SEX	UGI SCOPY	DUODENAL BIOPSY	BONE MARROW ASPIRATION	BONE MARROW BIOPSY
1	70	F	EROSIVE GASTRITIS	INTRA EPITHELIAL LYMPHOCYTOSIS	ERYTHROID HYPERPLASIA,MILD MEGALOBLASTIC	ERYTHROID HYPERPLASIA, NORMOBLASTIC MATURATION
2	36	M	GASTRIC POLYP	TROPICAL SPRUE/ENDOCRINE	MEGALOBLASTIC,INEFFECTIVE ERYTHROPOESIS	MEGALOBLASTIC
3	35	M	SCALLOPED DUODENAL FOLDS	TROPICAL SPRUE	#	#
4	65	F	SCALL D2	TROPICAL SPRUE	DIMORPHIC,PREDOMINANT MEGALOBLASTIC	#
5	74	F	SCALLOPED DUODENAL FOLDS	NONSPECIFIC CHRONIC DUODENITIS	#	#
6	45	F	EROSIVE GASTRITIS	BRUNNER GLAND HYPERPLASIA	#	#
7	55	F	#		#	#
8	62	M	SCALLOPED DUODENAL FOLDS	NO SPECIFIC PATHOLOGY	#	#
9	57	F	FLATTENED DUODENAL FOLDS	NO SPECIFIC PATHOLOGY	MEGALOBLASTIC	ERYTHROID HYPERPLASIA, NOEMOBLASTIC MATURATION
10	73	F	ATROPHIC GASTRIRIS	NO SPECIFIC PATHOLOGY	#	#
11	55	M	SCALLOPED DUODENAL FOLDS	TROPICAL SPRUE	HYPERCELLULAR,MEGALOBLASTIC	MEGALOBLASTIC
12	18	M	SCALLOPED DUODENAL FOLDS	NO SPECIFIC PATHOLOGY	MEGALOBLASTIC	MEGALOBLASTIC ERYTHROID HYPERPLASIA
13	57	F	#		#	#
14	71	M	#		#	#
15	48	M	#		#	#
16	25	F	SCALLOPED DUODENAL FOLDS	TROPICAL SPRUE	MEGALOBLASTIC ANEMIA WITH INEFFECTIVE ERYTHROPOESIS	ERYTHROID HYPERPLASIA, MEGALOBLASTIC MATURATION
17	17	F	#		MEGALOBLASTIC	MEGALOBLASTIC
18	75	F	#		#	#
19	45	M	SCALLOPED AND FLATTENED	NONSPECIFIC CHRONIC	#	#
20	87	M	#		#	#
21	54	M	#		#	#
22	46	M	#		MEGALOBLASTIC ANEMIA	ERYTHROID HYPERPLASIA
23	80	M	SCALLOPED DUODENAL FOLDS	CHRONIC DUODENITIS	#	#
24	48	F	#		#	#

#-not done

Master chart 2 continued

25	**54**	**M**	**#**		**#**	**#**
26	65	M	SCALLOPED DUODENAL FOLDS	TROPICAL SPRUE	#	#
27	55	M	#		#	#
28	58	M	SCALLOPED DUODENAL FOLDS	TROPICAL SPRUE	#	HYPERCELLULAR MARROW WITH
29	70	M	ATROPHIC GASTRIRIS	NO SPECIFIC PATHOLOGY	#	#
30	45	M	SCALLOPED DUODENAL FOLDS	TROPICAL SPRUE	MEGALOBLASTIC ANEMIA	MEGALOBLASTIC ANEMIA
31	60	M	#		MEGALOBLASTIC ANEMIA	#
32	70	M	SCALLOPED DUODENAL FOLDS	TROPICAL SPRUE	MEGALOBLASTIC ERYTHROID HYPERPLASIA	HYPERCELLULAR MARROW WITH MEGALOBLASTIC
33	24	M	SCALLOPED DUODENAL FOLDS	TROPICAL SPRUE	MEGALOBLASTIC ANEMIA	#
34	50	F	SCALLOPED DUODENAL FOLDS	INTRA EPITHELIAL LYMPHOCYTOSIS	MEGALOBLASTIC ANEMIA	#
35	65	F	SCALLOPED DUODENAL FOLDS	TROPICAL SPRUE	MEGALOBLASTIC ERYTHROID HYPERPLASIA	#
36	70	M	FLATTENED DUODENAL FOLDS	peptic duodinitis	MEGALOBLASTIC ANEMIA WITH	#
37	72	M	#		MEGALOBLASTIC ANEMIA	MEGALOBLASTIC ANEMIA
38	36	F	#		MEGALOBLASTIC ANEMIA WITH	MEGALOBLASTIC ANEMIA
39	58	F	SCALLOPED DUODENAL FOLDS	TROPICAL SPRUE	MEGALOBLASTIC ANEMIA	MEGALOBLASTIC ANEMIA
40	34	M	EXTRENSIC IMPRESSION BODY	TROPICAL SPRUE	#	#
41	48	M	SCALLOPED DUODENAL FOLDS	TROPICAL SPRUE	MEGALOBLASTIC ANEMIA	HYPERCELLULAR HYPERPLASIA
42	51	M	#		MEGALOBLASTIC ANEMIA	#
43	40	M	FLATTENED DUODENAL FOLDS	TROPICAL SPRUE	MEGALOBLASTIC ANEMIA	MEGALOBLASTIC ANEMIA
44	20	M	FLATTENED DUODENAL FOLDS	NO SPECIFIC PATHOLOGY	MEGALOBLASTIC ERYTHROID	#
45	58	M	ANTRAL GASTRITIS	NEURO	MEGALOBLASTIC ANEMIA	MEGALOBLASTIC ANEMIA
46	51	M	#	#	DIMORPHIC ANEMIA	DIMORPHIC ANEMIA
47	63	M	SCALLOPED DUODENAL FOLDS	TROPICAL SPRUE	MEGALOBLASTIC ANEMIA	#
48	60	M	SCALLOPED DUODENAL FOLDS	NONSPECIFIC CHRONIC DUODENITIS	MEGALOBLASTIC ANEMIA	MEGALOBLASTIC ANEMIA
49	59	M	SCALLOPED DUODENAL FOLDS	BRUNNER GLAND HYPERPLASIA	MEGALOBLASTIC ANEMIA	#
50	43	M	FLATTENED DUODENAL FOLDS	TROPICAL SPRUE	MEGALOBLASTIC ERYTHROID HYPERPLASIA	MEGALOBLASTIC ERYTHROID HYPERPLASIA

- Not done

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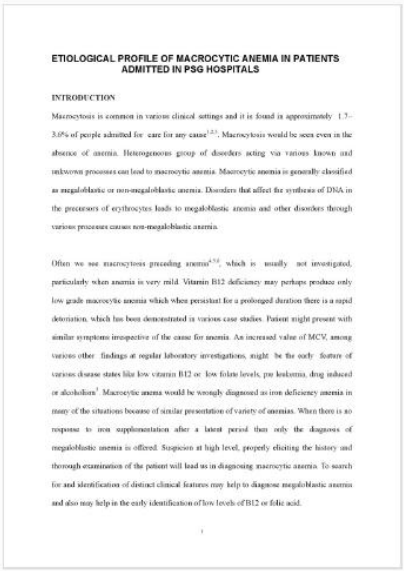

ETIOLOGICAL PROFILE OF MACROCYTIC ANEMIA IN PATIENTS ADMITTED IN PSG HOSPITALS

INTRODUCTION

Macrocytosis is common in various clinical settings and it is found in approximately 1.7–3.6% of people admitted for care for any cause[1,2]. Macrocytosis would be seen even in the absence of anemia. Heterogeneous group of disorders acting via various known and unknown processes can lead to macrocytic anemia. Macrocytic anemia is generally classified as megaloblastic or non-megaloblastic anemia. Disorders that affect the synthesis of DNA in the precursors of erythrocytes leads to megaloblastic anemia and other disorders through various processes causes non-megaloblastic anemia.

Often we see macrocytosis preceding anemia[4-7], which is usually not investigated, particularly when anemia is very mild. Vitamin B12 deficiency may perhaps produce only low grade macrocytic anemia which when persistant for a prolonged duration there is a rapid deterioation, which has been demonstrated in various case studies. Patient might present with similar symptoms irrespective of the cause for anemia. An increased value of MCV, among various other findings at regular laboratory investigations, might be the early feature of various disease states like low vitamin B12 or low folate levels, pre leukemia, drug induced or alcoholism[3]. Macrocytic anema would be wrongly diagnosed as iron deficiency anemia in many of the situations because of similar presentation of variety of anemias. When there is no response to iron supplementation after a latent period then only the diagnosis of megaloblastic anemia is offered. Suspicion at high level, properly eliciting the history and thorough examination of the patient will lead us in diagnosing macrocytic anemia. To search for and identification of distinct clinical features may help to diagnose megaloblastic anemia and also may help in the early identification of low levels of B12 or folic acid.

ETIOLOGICAL PROFILE OF MACROCYTIC ANEMIA IN PATIENTS

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