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To Study Hematological Features in Various Etiologies of Macrocytic Anemias

Authors
Mamatha G[1], Dr Shreelaxmi V Hegde[2], Dr Chethan Sagar[3], Dr Satheesha B C[4], Tintu Mathew[5]
[1]PhD-Research Scholar, Department of Biochemistry, Srinivas Institute of Allied Health Sciences, Science and Research Centre, Mangalore-575025, Karnataka, India
[2]PhD, Professor, Department of Biochemistry, Srinivas Institute of Medical Science and Research Centre, Mukka Mangalore-575025, Karnataka, India
[3]Associate Professor, Department of pathology, Subbaiah Institute of Medical Sciences and Research Hospital, Shivamoga, Karnataka, India
[4]Assistant Professor, Department of medicine, Subbaiah Institute of Medical Sciences and Research Hospital, Shivamoga
[5]Biostatistician, Subbaiah Institute of Medical Sciences, Shivamoga, Karnataka, India
Corresponding Author
Mamatha G
PhD-Research Scholar, Department of Biochemistry, Srinivas Institute of Allied Health Sciences, Mukka, Manglore-575025, Karnataka, India

Abstract
Background: *Mean corpuscular volume (MCV) increases by more than 100 fl. in macrocytosis, a condition in which erythrocytes are larger than normal. The purpose of this study was to evaluate the haematological characteristics in various aetiologies and to determine the underlying reasons of macrocytosis, which was found in regular hemograms. In addition to doing full blood counts, renal function tests, liver function tests, vitamin B12 assays, folate assays, and peripheral smear evaluations and few case were bone marrow examination.*
Material and Methods: *In this prospective observational study conducted over a period of one-year, medical records of 250 subjects were evaluated. Data was compiled of Megaloblastic, Non-megaloblastic Macrocytosis levels, along with clinical diagnosis and other biochemical parameters. Based on clinical evaluation and hematological and biochemeical evidence, patients were diagnosed as positive cases of Megaloblastic(166), Non-Megaloblastic (133).Among this two variable group Mean* ±SD *were also calculated .*
Results: *Compared to other age groups, male (83%) of 41-50 years ($p < 0.01$) were significantly affected as compared to female (16.4%) in similar age group. Our results showed , Vitamin B12 and Folic acid (156.47 ± 125.28, 3.24±4.61 respectively) for Megaloblastic and that for Non- megaloblatic was (400.24±66.35, 18.25±6.37 respectively).Categorical variables of Megaloblastic with Non-megaloblastic macrocytosis was tested using Independent samples t test, that showed a significant elevated ($p \leq 0.01$).among this Non megaloblastic macrocytes foused And analyzed the other parameters like Complete blood count, Peripheral smear and Biochemical parameters like RFT, LFT, were statistically distributed and calculated the Mean±SD .*
Conclusion: *Alcoholism, inadequate vitamin B12 intake, and drug use are the main causes of macrocytosis. Even if anaemia is not present, an elevated MCV needs to be examined because it can be the solitary sign of a pathological problem underneath.*
Keywords: *Macrocytosis: megaloblastic, Non-megaloblastic, Vitamin B12, Folic acid, LFT, RFT, Peripheral smear.*

Introduction

Red blood cells (RBCs) that are noticeably larger are referred to as macrocytosis. Mean corpuscular volume (MCV), a measurement of RBC size, is recorded in the complete blood count (CBC). The majority of reports of high MCV come with reports of elevated mean corpuscular haemoglobin (MCH). MCV and/or MCH elevations point to macrocytosis[1]. The presence of macrocytosis may not always call for additional testing or medical intervention. The significance of macrocytosis in the possible development of clinically significant anaemia (macrocytic anaemia) should be understood by practitioners, as well as the variety of pathologies that macrocytosis may indicate, frequently including extra-hematologic organ systems[2]. Finding the underlying aetiology is helpful in identifying the management solutions for patients with macrocytosis, which in turn mandates attention to history, physical exam, and laboratory results. Macrocytic anaemias have a wide range of causes, but they can be separated into two categories: megaloblastic anaemias and non-megaloblastic anaemias. Megaloblastic anaemias are traditionally classified by morphologic features seen during assessment of the peripheral blood smear[3,4]. Measurement of plasma or serum levels of vitamin B12 and folate as well as red cell folate is necessary for the evaluation of megaloblastic anaemia because vitamin B12/folate deficits cause a problem involving nuclear maturation[5]. A B12 level of less than 200 pg/ml (150 pmol/L) often indicates an underlying malabsorptive disease and is strongly suggestive of B12 insufficiency. In general, a B12 level of >400 pg/mL (300 pmol/L) excludes B12 insufficiency. Your body needs vitamin B12 as a necessary nutrient for functions like DNA synthesis, energy production, and central nervous system health[6]. According to studies, up to 20% of Americans and Britons over 60 may not be getting enough of this vitamin. Inadequate B12 levels can diminish the normal synthesis of red blood cells, which can compromise oxygen transport. This is frequently the result of restricted food intake, malabsorption, medical disorders, or the use of B12-depleting drugs[7].

Folate is a water-soluble vitamin that is naturally found in diet, particularly in fruits, green leafy vegetables, and liver. The synthetic version of folate found in fortified foods and supplements, folic acid has a better bioavailability than naturally occurring folate[8]. Folic acid insufficiency can be brought on by a number of factors, including insufficient dietary consumption. The folic acid is destroyed by heat during cooking. By active and passive transport mechanisms, folate is transported across the intestinal wall and absorbed in the jejunum. As a result, conditions like celiac disease, tropical sprue, short bowel syndrome, amyloidosis, gastric bypass, or mesenteric vascular insufficiency might prevent the absorption of folate, leading to a deficit[9]. Poor absorption of folate can also be caused by an elevated pH, such as that seen in achlorhydria. Drugs including methotrexate, phenytoin, sulfasalazine, and trimethoprim can impede the absorption of folate or convert it to its active form, which can cause a folate shortage. Due to methionine synthase dysfunction, which causes methylene THFA to accumulate in serum and cause the folate to be trapped as methyltetrahydrofolate and increase urinary folate excretion, folic acid deficiency can occur after vitamin B-12 deficiency. A big contributor to the lack of folate is alcoholism. Hemolytic anaemia, being pregnant, and other illnesses[10].

Causes of Megaloblastic Anemia Not Relate to B_{12}/Folate Deficiencies

Deficits in vitamin B12 and folate are other causes of megaloblastic anaemia. These drugs span a broad spectrum and either stop DNA synthesis or hinder the absorption, metabolism, or digestion of one or both vitamins. Methotrexate and other antifolates can cause functional folate shortage and megaloblastic changes. Alcoholism is associated with macrocytosis, which is directly harmful to the red cell membrane[11].

Causes of Megaloblastic Anemia

Folic acid and vitamin B12 are the major causes of megaloblastic anemia[12]. Folic acid and vitamin B12 deficiency leads to impaired DNA synthesis, ineffective erythropoiesis, and intramedullary hemolysis. Due to these condition the level of unconjugated bilirubin and serum LDH (lactate dehydrogenase) increased. LDH is a tissue marker breakdown and present in RBC. LDH released into circulation when destruction of RBCs occur. High level of LDH present due to tissue breakdown. High LDH level is one of the tool to identify the megaloblastic anemia. Increased serum LDH also seen in other condition for example in the case of hemolytic anemia[13]. Vitamin B12 deficiency also leads to thrombocytopenia in which reduction of platelet level to below normal. For the identification of Megaloblastic anemia bone marrow examination is necessary[14]. In megaloblastic anemia DNA synthesis is affected while RNA synthesis remain unaffected. Folic acid and vitamin B12 are necessary for the synthesis of purine and thymidylate, and their deficiency prevent DNA synthesis. Synthesis of DNA may be delayed when certain drugs are used such as purine antagonists, thymidylate antagonists and folate antagonist[15]. The inhibitors for reverse transcriptase interfere with DNA and cause macrocytosis. Most patients with HIV medication have macrocytosis without anemia. It means no treatment is necessary. The patients who have the evidence of blood loss, or increased cell destruction have macrocytosis. Myeloproliferative disorders is the common cause of macrocytosis in old people[16]. Macrocytosis also developed due to chronic obstructive pulmonary disease but it is not known whether the lung disease itself cause MCV to rise or some other diseases[17].

Causes of non-megaloblastic Anemia

Liver disease

Macrocytosis due to liver disease have cholesterol deposition on their RBC membranes. The reticulocyte (immature red blood cells) are 20% larger then mature red blood cells in macrocytosis [6]. Large erythrocytes or acanthocytes are developed with spike like projections and irregular surfaces and vary in length, width and distribution [18].

Alcohol Consumption

The people who are from nutritional deficiencies have impaired hematopoiesis when they intake alcohol. Chronic excessive alcoholism reduce the production of red blood cells and the erythrocytes that are larger than the normal. The red blood cells structure is also abnormal and the number of platelets also reduced. In people who use alcohol the size of RBC and MCV are larger than the people who does not use alcohol. However MCV alone is not appropriate to diagnose the non-megaloblastic anemia. The presence of large red blood cells (RBC) in blood is the indication of many other problems in addition to alcohol. For the identification of macrocytosis physician observe the blood under microscope to identify the structure of blood cells for each disorder. Thus the enlarged red blood cells in non-megaloblastic anemia are uniformly round while in case of megaloblastic anemia the red blood cell are oval in shape. People who drink excessive alcohol develop large RBC even in the absence of other factor that are responsible for RBC enlargement such as folic acid deficiency and alcohol liver disease. Alcohol abuse is a disorder mostly associated to macrocytosis, more than 80 % of men and 46% women have been found alcoholics [19]. The patients who have folate or vitamin B12 deficiency should obtain dietary education on the preparation and selection of food. Patients take diet that are rich in folic acid such as melons, bananas, lemon, lettuce, spinach, broccoli, asparagus and mushrooms. Foods should not be overcooked and not diluted in water. To avoid vitamin B12 deficiency dairy products and eggs must be included in diet. Patients must be know that there is a little quantity of folic acid

a. This study's objective is to categorise macrocytic anaemia into megaloblastic and non-

megaloblastic subtypes using haematological and biochemical indicators..

b. To evaluate the haematological features in different etiologies in macrocytic patient

Materials and Methods

This prospective observational study was performed from may 2022 to 2023, at the Department of Biochemistry, Maax superspecialty hospital, shimoga in India. The study included 250 adult patients with an MCV [100 fl]. New-borns, pregnant women, reticulocytosis and spurious macrocytosis were excluded from the study. All data of samples received for the analysis of CBC and peripheral smear and few bone marrow and biochemical parameters were included in the study after ethical clearance from the institutional ethic review board (IEC-SUIMS/36/Feb/23). Neonatal or children samples, hemolyzed, and samples were excluded as these interfere with the assay. Data for analysis from LIS and medical records was obtained after necessary clearance from IERB.. In all these cases, a complete medical history including diet, medication and alcohol intake was recorded. Sus tained and regular excessive drinking, alcohol tolerance and withdrawal symptoms were considered as 'Alcohoism'. All cases were subjected to serum Vitamin B12 (Cobalamin) assay, folic acid assay and liver function tests using fasting samples on Cases were subjected to serum Vitamin B12 (Cobalamin) assay, folic acid assay and using fasting samples on ADVIA CENTAUR XP, immunoassay system. Liver function tests were done using DIRUI CS-400, Auto-biochemistry analyser. A detailed evaluation of red cell parameters and peripheral smear was done in all cases. Based on the etiological factors identified, cases were segregated into megaloblastic and non-megaloblastic categories. Red cell parameters and peripheral smear findings were compared between these two categories. Bone marrow aspiration will done on few cases. The data obtained were entered along with patients details in excel sheet along with other biochemical parameters. Further the data were analyzed using SPSS version 18.The quantitative variables were analyzed with Chi square and t test.

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Results

Out of 250 population, Magaloblastic cases were 116 and Non megaloblastic 133.The both megaloblastic and non-megaloblastci consisted of 209 males (83.6%) and 41 females (16.4%) it shows statistically significant p <0.001 with the mean age of 49±15years (Table 1).

Table: 1 Age and Gender wise distribution

	Group		p-value
	Megaloblastic (116)	Nonmegloblastic (133)	
	Mean±SD		
Age	49.52±15.18	49.05±16.61	0.819
Gender	209(male)	41 (female)	<0.001

Table No: 2

There were 250 instances, 42 of which were megaloblastic with macrocytic anaemia (16.2%) and 74 of which were without anaemic (29.6%). In contrast, cases of non-megaloblastic, macrocytic anaemia were 45 (18.0%) and 89 (35.6%), respectively. Megaloblastic and non-megaloblastic patients without anaemia were 163 (65.2%), while cases with anaemia totaled 87 (34.8%).

Table: 2 Anemia and Non-anemic cases distribution

Group	Anemia	
	Yes	No
Megaloblastic (116)	42	74
Nonmegloblastic (133)	45	89
Total	87	163

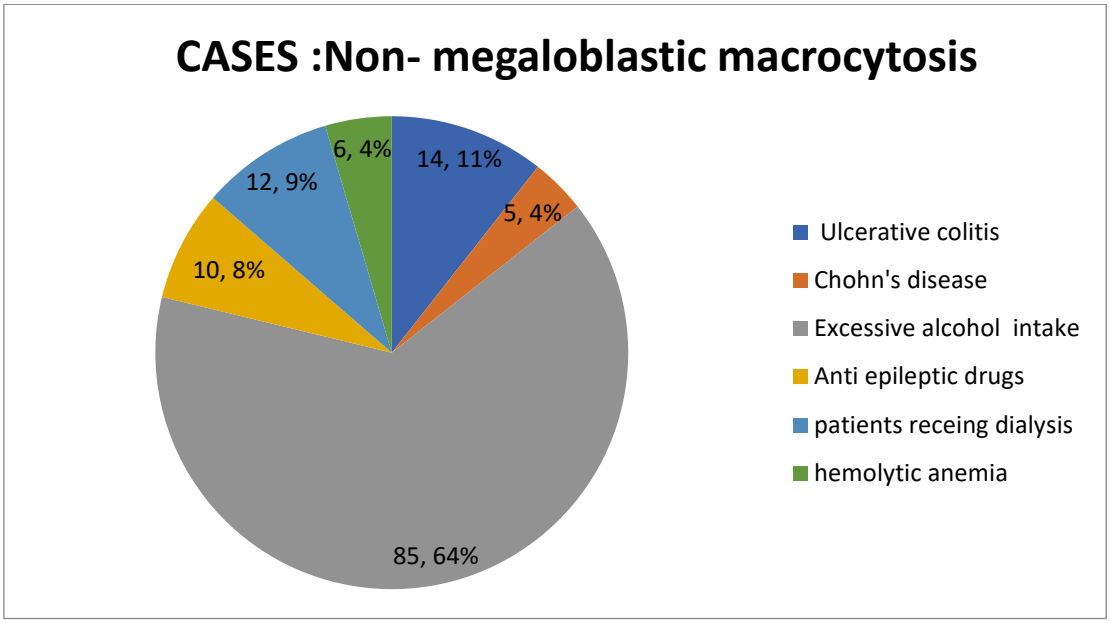
Table 3 mainly classified in both group considering hematological and biochemical parameter like Vitamin B12 & Folic acid, RFT , LFT, and Complete hemogram,

Variable	Group		p-value
	Megaloblastic	Nonmegloblastic	
	Mean±SD		
RBC	2.06±2.09	3.97±1.48	0.315
WBC	8540.08±3283.98	8183.92±3154.37	0.385
HB	13.20±2.96	15.28±14.65	0.134
PCV	39.27±8.78	40.19±9.48	0.427
MCV	104.75±4.41	104.31±4.23	0.426
MCH	34.57±2.63	34.74±2.37	0.600
MCHC	33.44±1.52	33.23±2.82	0.473
RDW	19.45±3.10	15.64±2.45	<0.003
ESR	32.46±26.50	30.72±28.90	0.628
Vitamin B12	156.47±125.28	400.24±66.35	<0.001
FOLIC ACID	3.24±4.61	18.25±6.37	<0.001
Creatinine	1.76±1.82	2.31±3.07	0.084
Uric acid	4.69±2.67	4.83±3.10	0.734
Total Bilirubin	1.64±2.27	2.92±2.80	0.220
Direct Bilirubin	0.83±1.07	0.76±0.87	0.624
Indirect Bilirubin	2.09±2.17	2.15±2.45	0.836
AST	57.25±63.12	71.16±80.61	0.161
ALT	49.42±71.31	67.04±82.33	0.096
ALP	112.67±110.60	139.37±138.07	0.230
GGT	63.32±70.25	162.3±170.01	0.003
Total Protein	6.53±0.72	6.52±0.85	0.965
Alb	3.52±0.54	3.42±0.62	0.223
A/G	1.26±0.57	1.32±0.53	0.390

Considered with biochemical test ,Vitamin B12(< 200) or folic acid (< 4) and few cases were bone marrow examination done based on that we categorized has Magaloblastic and Non-Megaloblastic macrocytosis. In both the group we compared Complete blood count, LFT and RFT test. Macroccytic anemia show in megaloblastic and absent in non –megaloblastic, RDW satirically significant in megaloblastic anemia cases. And show greater range of Vitamin b12 and folic acid value But there are other parameter we need to look forward like Creatinine frequency increased in non Magaloblastic and liver enzymes were increased in non megaloblastic anemia.

Non megaloblastic macrocytosis, and their clinical correlated Table:4

NON-MEGALOBLASTIC MACROCYTOSIS	CASES	PERCENTAGE (%)
Ulcerative colitis	14	10.7%
Chohn's disease	5	3.78%
Excessive alcohol intake	85	64.3%
Anti epileptic drugs	10	7.57%
patients receing dialysis	12	9.0%
hemolytic anemia	6	4.54%
Total No of cases	132	52.8%



Among the different etiology of Non megalobalstic macrocytosis, notify that excessive high risk alcohol intake 85 cases (64.3%) causing the macrocytic blood picture, 10.7(%) ulcerative colitis, Dialysis cases 9.0(%), anti epileptic drugs 10 (7.57%) hemolytic anemia 6 (4.54%), chohn's disease 5(3.78%) were analyzed in taken data and medical history from respective clinician.

Etiological factor/ factors could be identified in 132 (52.8%) as shown in Table 4. Alcoholism was the most common cause of Vitamin B12 deficiency found in 55 cases (72.6.1%) and drug related macrocytosis in 15 cases (19.8%). Alcoholism with liver diseases accounted for 12 cases (15.84%) of macrocytosis. Of the 16 cases diagnosed as Liver disease with kidney dysfuntion. As the clinicians opted to start combined Vitamin B12 and folate treatment, and there was no facility to perform serum homocysteine and methyl melanoic acid, we could not decide if the low cobalamin levels reflected actual 23 cases (12.9%) of drug-related macrocytosis and 43 cases (24.1%) of vitamin B12 insufficiency were found. These three infections were the main causes of macrocytosis in 73.6% of cases. Thirty-one instances had readings between 100 and 199 pg/ml, and 12 of the 43 cases that were determined to be vitamin B12 deficient had blood levels of 100 pg/ml. Three of these 12 patients had low folate levels, whereas nine of them were within the usual range. Bone marrow evaluation was done only when clinically indicated. Of the 10 cases, 7 cases revealed megaloblastic marrow, 1 cases appeared normoblastic and 1 cases showed dysplastic changes. Marrow iron stores were increased in two cases, of which one was MDS and the other was alcoholic liver disease.

Hematological parameters and blood smear findings observed in both these cataegories are compared in Table2.

The mean hemoglobin (Hb) was 8.2 g/dl in Vitamin B12/folate deficiency, as compared to 13.7 g/dl in alcoholism. The mean MCV was higher in megaloblastic cases. The maximum MCV observed in Vitamin B12 deficiency was 146.8 fl, while in alcoholism it was 113 fl. The mean RDW, an indicator of anisocytosis was significantly higher in the megaloblastic group. Megaloblastic anemia showed Macro-ovalocytes 75.4 % 17.2% Mean values are presented along with the range in brackets lower red cell counts, which was proportional to the degree of anemia. Peripheral smear in megaloblastic cases revealed hypersegmented neutrophils in 89.4% (32 cases) and macro-ovalocytes in 72% (27cases). In alcoholism, these features were found in 9.5 and 17.4% respectively. 82.6% cases of alcoholism

showed uniform, round mac- rocytes with normal RDW

Discussion

Our study shows Macrocytosis needs to be evaluated even in the absence of anemia, as it may be the first clue to an underlying pathology. Complete medical history including alcohol and drug intake, proper evaluation of red cell parameters and peripheral smear assist in arriving at a provisional diagnosis, thereby directing further management. This approach helps in determining the cause of macrocytosis, particularly in resource limited setting

In the various populations investigated, numerous causes of macrocytosis have been reported. The most common causes, according to Savage et al.'s[14] study of 300 hospitalized patients in a teaching hospital in New York City, were pharmacological therapy and alcohol addiction, while Vitamin B12/folate deficiency was infrequent (6%). Davidson et al.[7] also said that the most common cause was medicine[20].

According to Keenan et al.[15], the most common causes are haematological diseases and drinking. Their study did not include drug-induced causes, though. In their study of 124 patients between the ages of C75, Mahmoud et al.[16] discovered that macrocytosis is related with a higher risk of haematological disorders and malignancies. Breedveld and others[21].

According to Colon-Otero et al.[18], 60% of patients with macrocytosis present without anaemia. In the current study, 83 instances (46.7%) did not have anaemia, while 95 cases (53.3%) did and had anaemia as a co-morbidity. In our analysis, the relative incidence of anaemia varied among the various aetiologies, with Vitamin B12/folate insufficiency being the most common. Alcoholism and nonanemic macrocytosis were frequently linked[22].

Another crucial finding in this study was the isolated macrocytosis without anaemia that was seen in 9 (20.9%) patients of vitamin B12 insufficiency. This highlights once again how macrocytosis, which comes months before anaemia, might be a diagnostic forerunner of Vitamin B12 insufficiency. MCV is an average number that can be concealed by an iron-related concomitant microcytic anaemia, it must be highlighted[24].

The maximal MCV found in the current study was 146.8 fl for vitamin B12/folate insufficiency and 114 fl for alcoholism. The MCV is typically found to fall between 100 and 110 fl[6] in cases of alcoholism. The marrow erythroid precursors appear to be directly poisonous to ethanol. Due to the 120-day lifespan of erythrocytes, it may take 3–4 months for MCV to return to normal after quitting drinking[10]. In comparison to nonmegaloblastic circumstances (13.7%), the mean RDW (21.6%) in megaloblastic patients was significantly higher[25].

When comparing the red cell characteristics in macrocytosis between aplastic anaemia and megaloblastic anaemia, I Gupta and colleagues found that the RDW in the latter condition was significantly higher than the RDW in the former. Megaloblastic anaemia, myelodysplastic syndromes, and chemotherapy have all been linked to an elevated RDW [19,21]. RDW is normal or just slightly elevated in aplastic anaemia and liver illness[26].

Red cell characteristics in the macrocytosis of pernicious anaemia and alcohol misuse were compared by Seppa et al.[23]. The researchers discovered that a combination of low red cell count (4 9 1012/l), high RDW ([15%), normal thrombocyte count, and normal thrombocyte mean cellular volume was very sensitive in identifying Vitamin B12/folate deficiency. Bessmann et al.'s[27].

The presence of oval macrocytes (macro-ovalocytes) and hypersegmented neutrophils are characteristics of megaloblastic processes. However, in megaloblastic situations, hypersegmented neutrophils might occasionally be absent if they are connected to a pronounced left shift or severe neutropenia[27],[28] and Seppa et al. have reported Vitamin deficiency and alcoholism

as the most frequent etiological factors, which is similar to the findings in the present study.

Anticonvulsants (phenytoin, valproic acid), folate antagonists (methotrexate), chemotherapeutics (alkylating agents, pyrimidine and purine inhibitors), sulfasalazine (anti-inflammatory), pyrimethamine, trimethoprim, sulfamethoxazole, metformin, and nitrous oxide are among the medications that have been linked to macrocytosis. The majority of HIV patients on reverse transcriptase inhibitor therapy will exhibit macrocytosis without anaemia. Macrocytosis in these patients is used as a substitute marker for medication adherence in these patients.

Macro-ovalocytes are reported to be more sensitive but somewhat less specific than neutrophil hypersegmentation in predicting marrow megaloblastic changes[29]. It is reported that an MCV [115 fl, increased RDW, macro-ovalocytes, and hypersegmented neutrophils suggest a megaloblastic disorder. Nonmegaloblastic c3onditions display uniformly round macrocytes and normal RDW[3,30]. Round macro- cytes are commonly seen in alcoholism and round, target- appearing macrocytes are characteristic of liver disease such as hepatitis and obstructive jaundice. In the present study, hypersegmented neutrophils and macro-ovalocytes were more frequently observed in megaloblastic macrocytosis. However, these features were also observed in a small per-centage of alcoholism cases, who predominantly exhibited round, uniform macrocytes[31].

Conclusion

Vitamin B12 and folate are major factors of causing megaloblastic anemia. Inadequate intake of food, overcooking of food, and poor absorption cause Vitamin B12 and folate deficiency .The most frequent causes of macrocytosis include drinking, a lack of vitamin B12, and medicines. An elevated MCV needs to be evaluated even in the absence of anaemia because it can be the solitary sign of an underlying pathological condition. In the majority of instances, a comprehensive history that takes into account any medication and alcohol use, proper evaluation of the red cell characteristics, and a peripheral blood smear aid in the development of a preliminary diagnosis. Red cell characteristics and peripheral smears are regularly acquired and serve as straightforward, affordable, and useful methods for determining the cause of macrocytosis, particularly in resource-constrained locations.

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