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HEMATOLOGICAL STUDY OF MACROCYTIC ANEMIA: STUDY AT A TERTIARY CARE HOSPITAL

Pathology

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ABSTRACT

Introduction: Anemia is one of the most commonly diagnosed conditions in a health care set up. Iron deficiency anemia is the predominant cause of anemia across countries and in both sexes (1,2) . Although, in approximately 2 to 4 % of patients, laboratory evidence of macrocytosis is found (3) .The cause of macrocytic anemia is classified into megaloblastic or non-megaloblastic (4). Common causes of macrocytosis are different by region and setting. The present study aims at studying patients admitted in Civil Hospital Ahmedabad during the study period with MCV value more than 100 fL and utilizing the clinical profile and other biochemical parameters to further classify anemia into Megaloblastic and Non-Megaloblastic type. The study period is January 1, 2018 to July 31, 2019. **Materials and Methods:**Relevant medical history of the patients participating in the study was considered. MCV value was determined from complete hemogram which was performed using Automatic Hematological Analyser. Peripheral smear examination was done with slides stained in Giemsa stain. Biochemical tests were performed using Automated Biochemistry Analysers. The data obtained from the above was then utilized in establishing the incidence of various causes of macrocytic anemia in our study population. **Result:** Out of the total 325 patients 160 (49%) were found to have megaloblastic anemia and 128 patients (40%) were found to have non-megaloblastic anemia. In the remaining 37 (11%) patients, no cause of macrocytosis was identified. The etiologies of macrocytic anemia identified in the present study were Vitamin B12 deficiency, hepatic dysfunction, hypothyroidism, renal dysfunction and accelerated erythropoiesis (due to hemolysis or blood loss). **Conclusion:** The most common cause of macrocytic anemia in our study population was found to be Megaloblastic anemia.

KEYWORDS

Anemia, Macrocytic, Megaloblastic, Non-Megaloblastic.

INTRODUCTION

The World Health Organization (WHO) defines anemia as hemoglobin (Hb) count of less than 13 g/L in men, less than 12 g/L in non-pregnant woman, and less than 11 g/L in pregnant women and the elderly [2].In 2010, a global anemia prevalence was 32.9%, that is, more than 2.2 billion people were affected [2]. The cause of anemia varies by age, sex, and geography, and iron-deficiency anemia is the most common etiology [2], although macrocytic anemia is regularly encountered in general practice and in hospital settings.

The mean corpuscular volume (MCV) is calculated from hematocrit (%) x 10/RBC count (10^6 /microL) [5]. Normal MCV values range from 80 to 100 femtoliters (fl) in adults [5]. In 1.7% to 3.6% of cases involving patients seeking medical care, MCV is increased, often in the absence of anemia [6,5,7,8]. Mild macrocytosis (MCV of 100 to 110 fl) is particularly common and often remains unexplained in even after careful study**.** Even so, this finding should not be ignored, because it can be an important early clue to a reversible process. For example, it may appear 1 year or more before anemia develops in patients with pernicious anemia, and neurologic disease can progress during that interval [9].

The cause of macrocytic anemia is classified into megaloblastic or non-megaloblastic [10]. Megaloblastic anemia is caused by deficiency or impaired utilization of vitamin B12 and/or folate, whereas non-megaloblastic macrocytic anemia is caused by various diseases such as myelodysplastic syndrome (MDS), liver dysfunction, alcoholism, hypothyroidism, certain drugs, and by less commonly inherited disorders of DNA synthesis [10]. Common causes of macrocytosis are different by region and setting.

No complications arise from macrocytosis itself as an isolated finding [11]. However, its identification can provide important information regarding the presence of an underlying disease state [11].

Materials and methods

This study was a prospective observational study of all patients with MCV value more than 100 fL, admitted in Civil Hospital Ahmedabad during the study period. A total of 325 patients were studied. Relevant medical history of the patients participating in the study was considered. MCV value was determined from complete hemogram which was performed using Horiba pentra XLR automatic Hematological analyzer (five part differential) installed at the Hematology section of Pathology department, Civil Hospital, Ahmedabad. For this purpose, Peripheral blood was drawn from all study subjects under aseptic precautions in EDTA vacutainer. Peripheral smear examination was done with slides stained in Giemsa stain. Further work up of macrocytosis was aimed at classifying it into megaloblastic and non-megaloblastic types by determining its causal factors utilizing relevant medical history and other biochemical parameters like Serum Vitamin B12 assay, Liver function tests, Renal function tests and Thyroid function tests. Liver function tests and Renal function tests were performed using Erba XL-640 Automatic biochemistry analyser installed at Biochemistry laboratory, Civil Hospital, Ahmedabad. Serum Vitamin B12 assay and Thyroid function tests were performed using Beckman Coulter Unicel DxI 600- access immunoassay system. The data obtained from the above was then utilized in establishing the prevalence of various causes of macrocytic anemia in the present study population. Data analysis was done with using Microsoft excel 2013 and SPSS (Statistical Package for the Social Sciences).

Ethical considerations

All procedures performed were in accordance with the ethical standards of the institution.

Observations and Results

Present study population consisted of total 325 patients, out of which 220 were males comprising about 68% and 105 were females comprising about 32%. Majority of the population belonged to the age group 31-40 years in both males and females.

In our study, 160 patients suffered from Vitamin B12 deficiency, 133 patients had liver dysfunction, 26 patients had hypothyroidism, 19 patients had renal dysfunction, and 5 patients had accelerated erythropoiesis. In 1 case, macrocytic anemia was observed in a patient diagnosed with possibility of Chronic Lymphoproliferative disorder (CLPD).

Out of the 160 Vitamin B12 deficient patients, 27 patients also had concomitant hepatic dysfunction, 8 patients had concomitant hypothyroidism and 3 patients had concomitant renal dysfunction as evidenced by relevant history and biochemical parameters.

There were 37 such patients in whom macrocytic anemia was established by haemoglobin level and peripheral blood smear examination but either no relevant history or biochemical evidence was found to indicate a definitive cause of macrocytosis or further

work up as to the cause of macrocytosis could not be performed.

Out of the total 325 patients, 293 patients had given a negative history of alcohol consumption, whereas, 32 patients had given a positive history of alcohol consumption out of which 28 patients suffered from liver dysfunction as evidenced by abnormal liver function tests.

In our study, out of the total 325 patients, 93% patients (n=302) were vegetarians and 7% patients (n=23) had non-vegetarian diet.

Out of the total 325 patients, 160 patients had Megaloblastic anemia. Out of these 160 patients, 153 patients gave history of vegetarianism and 7 patients gave history of consumption of mixed diet.

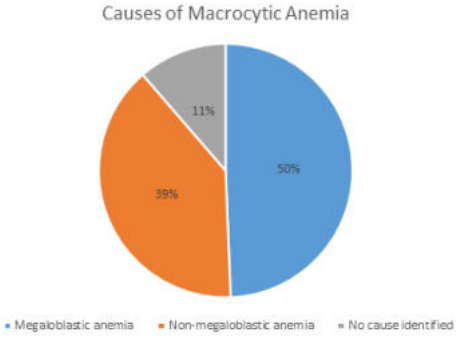

CHART I: CAUSES OF MACROCYTIC ANEMIA

Thus, the most common cause of macrocytic anemia in our study is Megaloblastic anemia.

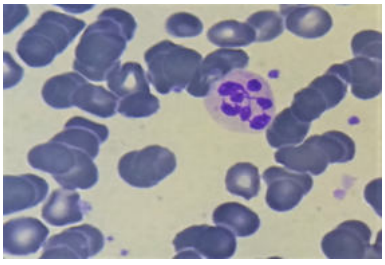

FIGURE I: PS FINDING IN OUR PATIENT - HYPERSEGMENTED NEUTROPHIL (7 LOBES), (GEIMSA STAIN, MAGNIFICATION - 100X)

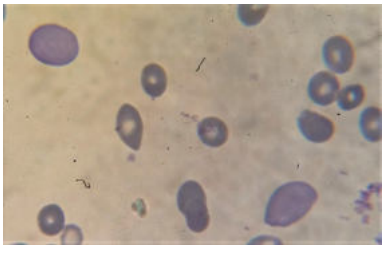

FIGURE II: PS FINDING IN OUR PATIENT - POLYCHROMATIC RBCs, (GEIMSA STAIN, MAGNIFICATION - 100X)

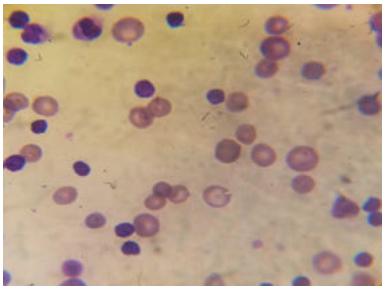

FIGURE III: PS FINDING IN OUR PATIENT - MACROCYTES IN CHRONIC LYMPHOPROLIFERATIVE DISORDER, (GEIMSA STAIN, MAGNIFICATION - 100X)

DISCUSSION

In our study population, most common cause of macrocytic anemia was found to be Vitamin B12 deficiency (n=160, 49%). The second commonest cause of macrocytic anemia in our study was found to be hepatic dysfunction (n=133, 41%). In comparison, the most common cause of macrocytic anemia in other studies is shown in table I.

TABLE I: MOST COMMON CAUSE OF MACROCYTIC ANEMIA IN VARIOUS STUDIES.

STUDY	COMMON CAUSE IDENTIFIED
Our study	Megaloblastic anemia
Colon-Otero et al [12]	Alcohol abuse
McPhedran et al [8]	Megaloblastic anemia
Chidambaram Y et al [13]	Megaloblastic anemia

Comparing gender ratio with other studies, Chidambaram Y et al and Vineetha Unnikrishnan et al also had more male : female ratio as that of our study [13,14]. But studies done by Tejas Shah et al and Uma Khanduri et al showed female predominance [15,16].

In our study 4% of the patients with vitamin B12 deficiency were on non-vegetarian diet and 96% were on vegetarian diet. In concordance with our study, other studies conducted by Liggy et al and SR Kankonkar et al showed that vitamin B12 deficiency is higher in vegetarians compared to non-vegetarians [17,18]. In contrast to our study, Chidambaram Y et al showed Vitamin B12 deficiency in 91% and Iqbal SP et al showed Vitamin B12 deficiency in 94% of non-vegetarians [13, 19].

In our study, out of the total study population of 325, hypersegmentation of neutrophils was found in peripheral smears of 128(39.4%) patients out of which 125(38.5%) had Megaloblastic anemia and macro-ovalocyte was found in peripheral smears of 62(19.1%) patients out of which 51(15.7%) had Megaloblastic anemia.

TABLE II: COMPARISON OF PERCENTAGE OF PATIENTS WITH MEGALOBLASTIC ANEMIA HAVING HYPERSEGMENTATION OF NEUTROPHILS IN THEIR PERIPHERAL SMEARS.

STUDY	PERCENTAGE
Our study	80%
Chidambaram Y et al [13]	50%
Khanduri and Sharma [16]	100%
Vineetha Unnikrishnan et al [17]	43%

The following tables show association of various haematological parameters with increased MCV and possible etiologies respectively.

TABLE III: ASSOCIATION OF VARIOUS HEMATOLOGICAL PARAMETERS WITH INCREASED MCV

Test of association applied	Hematological parameter	p value*	n	Test value
Pearson correlation test	Hemoglobin (gm/dl)	0.096	325	-0.92
Pearson correlation test	Total leucocyte count (cells/cumm)	0.004	325	0.159
Pearson correlation test	RDW	0.000	325	-0.470
Pearson correlation test	Platelets (cells/cumm)	0.115	325	0.088
Pearson correlation test	TSH (uIU/ml)	0.957	112	-0.005
Pearson correlation test	SGPT (IU/L)	0.344	218	-0.064
Pearson correlation test	SGOT (IU/L)	0.660	157	-0.35
Pearson correlation test	ALP (U/L)	0.549	158	0.48
Pearson correlation test	Sr.Vitamin B12 (pg/ml)	0.722	198	0.026
Pearson correlation test	Sr. Creatinine (mg/dl)	0.342	119	-0.088

TABLE IV: ASSOCIATION OF HEMATOLOGICAL PARAMETERS WITH VARIOUS POSSIBLE CAUSES OF ETIOLOGY.

Test of association applied	Hematological parameter	p value*	Test value
Anova test	Hemoglobin (gm/dl)	0.112	2.205

Anova test	Total leucocyte count (cells/cumm)	0.093	2.391
Anova test	MCV (fl)	0.644	0.441
Anova test	MCH (pg)	0.212	1.559
Anova test	MCHC (gm/dl)	0.841	0.173
Anova test	RDW	0.451	0.799
Anova test	Platelets (cells/cumm)	0.096	2.357
Anova test	Reticulocyte count (%)	0.024	3.393
Anova test	TSH (uIU/ml)	0.094	2.414
Anova test	SGPT (IU/L)	0.021	3.928
Anova test	SGOT (IU/L)	0.000	8.166
Anova test	ALP (U/L)	0.000	21.881
Anova test	Sr. Vitamin B12 (pg/ml)	0.000	8.209
Anova test	Sr. Creatinine (mg/dl)	0.089	2.473
Fisher's exact test	Hypersegmentation of neutrophils	0.000	225.62
Fisher's exact test	Macro-ovalocyte	0.000	33.915

(* - p value < 0.05 is statistically significant)

Thus, from the above, it is observed that, when MCV is compared to the values of hemoglobin, using Pearson correlation test, no association was found (p value > 0.05). This observation is similar to the observation in the study done by Chidambaram et al. [13].

And, when Serum vitamin B12 levels are compared with MCV values, using Pearson correlation test, no association was found (p value > 0.05).

This observation was similar to the observation in the study done by Chidambaram et al [60-13].

In our study, hypersegmented neutrophils were present in peripheral smears of 125(38.5%) patients with Megaloblastic anemia, 2(0.6%) patients with Non-Megaloblastic anemia and in 1(0.3%) patient in which no etiology was identified. Also, Macro-ovalocytes were present in 51(15.7%) patients with Megaloblastic anemia, 9(2.8%) patients with Non-Megaloblastic anemia and in 2(0.6%) patients in which no etiology was identified. Thus, it was observed that, larger number of patients with hypersegmented neutrophils and macro-ovalocytes had a Megaloblastic cause of anemia. Using the test of association (Fisher's exact test) between hypersegmentation of neutrophils with etiology and also, macro-ovalocytes with etiology, we observe a p value <0.05, which is significant. Thus, showing a positive association of the above two peripheral smear findings with the etiology of megaloblastic anemia.

Limitations of the study

The following were 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 who did not have anemia might have been missed. So, the actual prevalence of vitamin B12 and folate deficiency could not be identified from this study.
2. Serum Folate or red cell folate levels could not be done due to unavailability of the required resources for performance of the tests.
3. Bone marrow examinations (for megaloblastic anemia) were not performed in any of the patients.
4. In patients presenting 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.

CONCLUSIONS

1. Although there are many etiologies of macrocytic anemia, in our study Vitamin B12 deficiency (causing Megaloblastic anemia) was the most common etiology.
2. Hypersegmented neutrophils and macro-ovalocytes occurring in peripheral blood smear would significantly favour towards diagnosing megaloblastic anemia.
3. In our study, the majority of patients with Vitamin B12 deficiency are vegetarians, and a small minority of patients had a mixed diet. So, it can be concluded that even though vegetarianism did cause patients to be more prone to vitamin B12 deficiency, in a minority of patients, non-vegetarianism did not confer protection against it.
4. There were a number of patients in whom no cause of macrocytosis could be established, with the investigations used in the study, which demonstrates the diversity of causes of macrocytic anemia and hence the need of wider panel of investigations required to support the etiologic causes.

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