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The Diagnostic Value and Sensitivity of Blood Film in diagnosis of Megaloblastic Anemia

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Abstract: **Background:** Megaloblastic anemia is a type of macrocytic anemia primarily caused by vitamin B12 and/or folate deficiency. Early diagnosis is crucial to prevent complications, particularly neurological impairments associated with vitamin B12 deficiency. Blood film examination is a cost-effective diagnostic tool for identifying macrocytic anemia. **Objective:** This study evaluates the diagnostic value and sensitivity of blood film in detecting Megaloblastic anemia and its association with vitamin B12 deficiency. **Methods:** A retrospective cross-sectional observational study was conducted on 31 patients diagnosed with macrocytic anemia using blood film. Laboratory investigations included complete blood count (CBC), red blood cell indices, and serum vitamin B12 levels. Data analysis was performed using statistical software, with significance set at $p < 0.05$. **Results:** The median age of patients was 65 years, with 71% being female. The mean hemoglobin level was 7.52 g/dL. Among the patients, 67.7% had macrocytic anemia, while 32.3% had non-macrocytic anemia. A highly significant association was found between macrocytic and vitamin B12 deficiency ($p = 0.035$). Multivariate analysis confirmed that low vitamin B12 levels were a strong predictor of macrocytic anemia ($p = 0.048$). **Conclusion:** Blood film examination is a sensitive and reliable method for diagnosing macrocytic anemia. The study highlights the strong correlation between vitamin B12 deficiency and macrocytosis, reinforcing the importance of routine screening for vitamin B12 levels in anemic patients.

Keywords: Megaloblastic anemia, Macrocytic anemia, Vitamin B12 deficiency, Folate deficiency, Macrocytosis, CBC.

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INTRODUCTION

Megaloblastic anemia is a form of macrocytic anemia. Macrocytic anemia is a blood disorder that causes by vitamin B12 and/or vitamin B9 (folate) deficiency [1]. Vitamin B12 deficiency can be cause by Dietary deficiency [6], Lack of intrinsic factor (IF) as (Pernicious anemia and Gastrectomy, Biologic competition for B12 as over growth of bacteria (Blind loop syndrome, Fish tapeworm infestation, also by Pancreatic insufficiency, and it rare cause by Zollinger-Ellison syndrome [2].

The vitamin B9 (folate) deficiency can occur with diet doesn't include green vegetables, fruits. Also it has other causes may include digestive system diseases as Crohn's disease or celiac diseases, excessive alcohol use [8], hemolytic anemia, and some drugs as anti - seizure drugs and ulcerative colitis drugs [7] interfere with the proper absorption of folate [3].

Megaloblastic anemia symptoms are similar to symptoms of other types of anemia, fatigue, weakness, pallor, shortness of breath (dyspnea) [9].

Vitamin B12 deficiency sometimes affects your nerves [4], causing symptoms like tingling sensations, loss of sensation or muscle weakness [10].

Diagnosis of macrocytic anemia detected by chemical analyze of Complete blood count (CBC), RBC indices, reticulocyte count, and peripheral blood smear, B12 and folate levels [13].

METHODS

Retrospective cross –section observational study .A sample of patients was selected from the laboratory department outpatient clinic.

Study design and sample size: thirty-two AS patients were included in the study, age and sex match in this study .All patients were diagnosed macrocytic anemia by blood film.

Individuals with all patients above 18 years old in were diagnosed as macrocytic anemia by blood film in

tobruk city in 2024, neonate, patients below 18 years old were excluded.

The local Ethics committee of faculty of medicine-Tobruk University approved the study protocol. Before enrolling in the study, the laboratory signed an informed consent form.

The sample size was determined using data from result of blood film .This retrospective cross- section study was determined by macrocytic anemia in blood film.

Techniques

Peripheral Blood Smear, A drop of blood is applied against a glass slide that is subsequently stained with polychrome stains (Wright-Giemsa) to permit identification of the various cell types. These stains are mixtures of basic dyes (methylene blue) that stain as blue and acidic dyes (eosin) that stain as red. Acid components of the cell (nucleus, basophilic granules) stain blue or purple, and basic components of the cell (hemoglobin, eosinophilic granules) stain red. Peripheral smear description Macrocytosis and Anisopoikilocytosis [12].

Laboratory tests

The blood sample were collected subject to chemical analysis, the coulter counter mode was used to analyze Complete blood cell (CBC), which include the hemoglobin (HB), total white blood cells (WBCs), Platelets counts, MCV, also used Vitamin B 12 level in blood [5].

Statistical analysis

Data management- analysis- and graphs were performed using R Studio software version 4.4.1. Numerical data were summarized using means and standard deviations or medians and/or interquartile ranges- as appropriate. Categorical data were summarized as numbers and percentages. Estimates of the frequency were done using the numbers and percentages. Numerical data were explored for normality using Kolmogrov-Smirnov test and Shapiro-Wilk test. Chi square or Fisher's tests were used to compare between the independent groups with respect to categorical data- as appropriate.

Multinomial logistic regression was used to estimate the association between cases with and without vitamin B12 deficiency and clinical parameters, knowing that cases without macrocytic category were used as the reference level.

Comparisons between two groups for normally distributed numeric variables were done using the Student's t-test while for non-normally distributed numeric variables- comparisons were done by Mann-Whitney test. Statistical significance was established as a $P < 0.05$.

RESULTS

The study included 31 patient, the median age was 65 years (ranging from 49.5 to 70.5 years 71%) were females, while 29% were males). The mean hemoglobin (HB) level was 7.52 g/d), %67 of patients had macrocytic red blood cells, while 32.3% had non-macrocytic cells (The median platelet (PLTs) count was 78,000/µL. The median white blood cell (WBCs) count was 4,000/µL. The median vitamin B12 level was 120 g/mL, indicating that most patients had vitamin B12 deficiency.

Table 2 shows the Demographic Data of Patients With and Without Macrocytic Anemia, This table compares patients who have macrocytic anemia (21 patients) with those who do not (10 patients) based on age and gender

Age, The median age of macrocytic patients was 67 years (range: 42-72).
The median age of non-macrocytic patients was 62 years (range: 55.5-69).
P-value = 0.816, indicating no significant difference in age between the two groups.
Gender, Among macrocytic patients: 66.7% were females, and 33.3% were males.
Among non-macrocytic patients: 80% were females, and 20% were males.
P-value = 0.733, meaning gender was not significantly different between the groups.

Table 3 examines the relationship between Mean Corpuscular Volume (MCV) and macrocytic in blood film

All 21 macrocytic patients had macrocytic (100%), while none of the non-macrocytic patients did P-value < 0.001, showing a highly significant difference.

Vitamin B12 levels were significantly lower in macrocytic patients ($p = 0.035$), suggesting that vitamin B12 deficiency is strongly associated with macrocytic.

Table 4, analyzes the influence of different factors on MCV using Odds Ratio (OR) and Confidence Interval (Ci). This confirms that low vitamin B12 levels are a strong predictor of macrocytic anemia, while other factors (age, gender, HB, platelets, and WBCs) do not significantly affect MCV. Only vitamin B12 levels showed a statistically significant association with macrocytic ($p = 0.048$).

Table-1: Descriptive data regarding macrocytic anemia

		Overall (n =31)
Age (median (IQR))		65 (49.5- 70.5)
Gender (%)	Female	22 (71)
	Male	9 (29)
HB (mean (SD))		7.52 (1.67)
mcv (%)	Macrocytic	21 (67.7)
	Non macrocytic	10 (32.3)
plts (median (IQR))		78 (30-180)
wbcs (median (IQR))		4 (2.8-8)
vit.b12 (median (IQR))		120 (71.5-607)

Data are expressed as n (%), mean (SD), and median (IQR).

Table-2: Demographic Data of patients with and without macrocytic anemia

		Macrocytic (n= 21)	**Non macrocytic (n=10)**	***P***
Age (median (IQR))		67 (42- 72)	62 (55.50- 69)	0.816
Gender (%)	Female	14 (66.7)	8 (80)	0.733
	Male	7 (33.3)	2 (20)	

Data are expressed as n (%) and median (IQR). *: Statistically significant at P < 0.05.

Table-3: The association between MCV and clinical parameters

		Macrocytic (n= 21)	**Non macrocytic (n=10)**	***P***
HB (mean (SD))		7.46 (1.61)	7.65 (1.86)	0.774
mcv (%)	Macrocytic	21 (100)	0 (0)	<0.001*
	Non macrocytic	0 (0)	10 (100)	
plts (median (IQR))		100 (30- 190)	60.5 (29.25- 77.5)	0.228
wbcs (median (IQR))		4 (3- 8)	3.75 (2.15- 7.05)	0.566
vit.b12 (median (IQR))		78 (53- 400)	549 (176.25- 725.50)	0.035*

Data are expressed as n (%), mean (SD), and median (IQR). *: Statistically significant at P < 0.05.

Table-4: Multivariate logistic regression of clinical parameters with MCV

Characteristic	**OR**[1]	**95% CI**[1]	**p-value**
Gender			
Female	—	—	
Male	0.25	0.02- 2.03	0.2
Age	1.02	0.97- 1.09	0.5
HB	1.23	0.66- 2.62	0.5
plts	0.99	0.98- 1.00	0.2
wbcs	1.02	0.96- 1.20	0.7
vit.b12	1.00	1.00- 1.01	0.048*

[1] OR = Odds Ratio, CI = Confidence Interval. *: Statistically significant at P < 0.05.

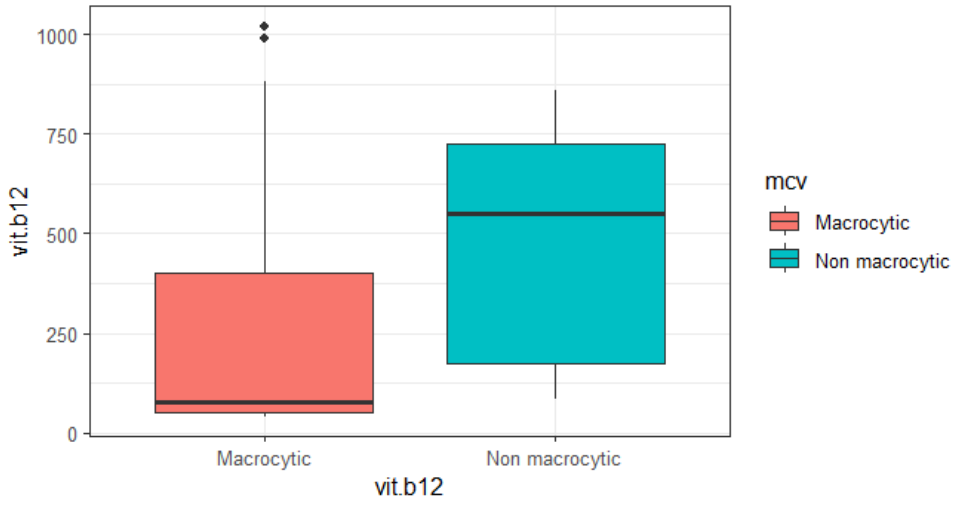

Fig-1: The box plot for the association between MCV and vitamin B12

DISCUSSION

The goal of this study to evaluate the sensitivity of blood film in diagnosis of Megaloblastic anemia.

The result of study revealed no significant difference in age between the two groups macrocytic patients and non-macrocytic patients, but on the other side, Takayo Nagao *et al*., which found incidence macrocytic anemia increase in elderly patients, which can explain by has a large number in his study [16]. Also gender was not significantly different between macrocytic patients and non –macrocytic patients.

But there was a highly significant difference the relationship between Mean Corpuscular Volume (MCV) and macrocytic in blood film. All 21 macrocytic patients had macrocytic (100%), while none of the non-macrocytic patients did P-value < 0.001, ….Vineentha U *et al*, found macrocytic anemia was identified by blood film and increase mean corpuscular volume (MCV>100) [20]. Currently, Blood film and MCV are commonly used as diagnosis macrocytic anemia patients.

Also, in our study, Vitamin B12 levels were significantly lower in macrocytic in blood film (p = 0.035), suggesting that vitamin B12 deficiency is strongly associated with macrocytic. Similarly, Fuad A *et al.*, which found correlation between vitamin B12 deficiency and macrocytic anemia [14] .Additionally, S.N wickramasinghe *et al*., reported there was a strong relationship between vitamin B12 deficiency and macrocytic anemia [15]. Also, many studies, (A gupta *et al.*, Ghulam Shah *et al.*, Vineentha U *et al*. --) have indicated that there in line with this study. All of their study found a strong relationship between vitamin B12 deficiency and macrocytic anemia patients [17,18,20].

The current study showed the sensitivity blood film in diagnosis Megaloblastic anemia in patient with high mean volume corpuscular (MCV) in patient with low vitamin B 12 level, which the same results found in the study of Olger Nano *et al.* was blood film identify Megaloblastic anemia [19], similarly, Assad babker *et al*., which was Identification of megaloblastic anemia cells through the use of image processing techniques of blood film [21].

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