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RESEARCH PAPER

Diagnosis and Differentiation of Macrocytic Anemia in Anemic Patients of Erbil Governorate

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A B S T R A C T:

Macrocytic anemia (MA) is an increase in mean corpuscular hemoglobin (MCH) concentrations, mean cell volume (MCV), and red blood cell dispersion width (RDW). Insufficiencies in vitamin B12 and folate are the most frequent sources of macrocytic anemia the objective of cross-sectional research were conducted to diagnose and differentiate anemic patients in Erbil Governorate. The study had 162 patients in total, of which 50 were healthy and 112 had been given a Macrocytic anemia diagnosis. Three groups of research participants were created; 50 healthy subjects from Group 1, 33 Megaloblastic anemia patients from Group 2, and 79 non- megaloblastic patients from Group 3 were included. Complete blood count (CBC), Reticulocyte count, T3, T4, TSH, Ferritin, B12 and Folic acid. The findings showed that the MCV, MCH, and RDW in MMA and non-MMA subjects all had increased significantly ($P<0.0001$). Both Megaloblastic and non-Megaloblastic group demonstrated a considerable reduction in HB, HCT, RBC count, and PLT count. The results showed a very high significant in serum ferritin ($P< 0.0001$), but a significant reduction in both folic acid and vitamin B12 MMA. In both MMA and non-MMA individuals, thyroid hormone levels (T3) did not alter significantly. although there were a considerable difference in T4 and (TSH) in MMA and non-MMA.

KEY WORDS: Megaloblastic Anemia, Non-Megaloblastic Anemia, VitaminB12&Folate insufficiency, Alcoholism, Hypothyroidism
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1. INTRODUCTION :

Anemia is characterized by a decline in red blood cell count per cubic millimeter (RBCs), hematocrit, or hemoglobin concentration (Hb), The results of present of Hb or (Hct) is less than the normal range, Anemia is determined by morphology(MCV), and red cell dispersion breadth (RDW) (Chulilla et al., 2009).

The requirements of the World Health Organization (WHO) is12 gm/dL for anemia in women and 13 gm/dL in males. However, it varies based on your age, sex, and pregnancy status (Ismailetal.,2017).

and Mean cell volume (MCV) make up the red cell indices, which describe the Hb content and size of the RBC. RBC indices are used to identify and classify different kinds of anemia. The RBC deficiency can be categorized as macrocytic, normocytic, or microcytic cells, or as microcytic hypochromic cells, depending on cell size (Kareem and Mohammed, 2022).

Megaloblasticerythropoiesis,hypersegmentation of neutrophil nuclei, pernicious anemia are all signs of B12 insufficiency anemia, as are morphological abnormalities of various hematological growths in the bone marrow (Makhmonov et al., 2021)

The cause of MMA is B12 deficiency which largely affected on elderly persons (Green, 2017). The first most typical reason for anemia is a dietary insufficiency folic acid or vitamin B12(Moll and Davis, 2017). A common symptom of vitamin B12 and folate insufficiency is

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macrocytic anemia, The elevation in MCV can be used to make the diagnosis of B12 deficiency (Smelt et al., 2018)

For the DNA synthesis Vitamin B12 which essential for cell division and proliferation, Its deficiency causes psychiatric and hematological disorders and physical and neuromotor growth retardation, as well as megaloblastic anemia. Especially due to its demyelination effect, brain and nervous system developments are significantly affected (Buke et al., 2020)

The production of RBC required folic acid and the shortage of it cause folate deficiency anemia(Obeagu et al., 2021). Low folic acid levels might result in megaloblastic anemia(Castellanos-Sinco et al., 2015). Red blood cells with this abnormality became larger than normal, fewer in number, and oval-shaped rather than spherical. (Bhadra and Deb, 2020) Folate is essential for the production of DNA and for hematological activities; its absence can result in a variety of conditions, including megaloblastic anemia. (Green and Mitra, 2017)

Non-megaloblastic anemia can be brought on by a wide range of conditions, including hypothyroidism, myelodysplastic syndrome (MDS), alcoholism, and liver disease. Geographical and environmental factors affect the most frequent causes of macrocytosis. (Single,. etal.2022). The main factor causing macrocytosis was drunkenness. (Dasgupta, 2015) .

To differential of macrocytic anemia to megaloblastic and non- megaloblastic anemia. And to determine the causes of macrocytic anemia to megaloblastic and non-megaloblastic in Erbil Governorate.

2. MATERIALS AND METHODS

In the current study include 70 men and 42 women made up the 112 subjects. The age range of the subjects was greater than 30. All patients were diagnosed with MA, which was further divided into 79 patients (50 males and 29 females) with non-MMA and 33 patients with MMA (20 men and 13 females) 50 patients made comprised the control group (27 males and 23females). Samples were collected from Erbil Governorate in Kurdistan Region, Iraq. The research was done between October 2021 and March 2022.

2.1 Collection of the samples

Before collecting blood samples, participants are requested to complete a unique questionnaire, which is then filled up by conducting a direct or indirect conversation with patients from several hospitals in the Erbil Governorate**.** Using a sterile disposable syringe, five ml of blood was drawn by vein puncture from patients and control volunteers. Two tubes of blood were used to separate the samples: one tube contained 2 ml of an anticoagulant containing EDTA, which was processed for hematological analysis within six hours; 3 milliliters of blood were present in the other tube and were permitted to clot. To obtain serum for ferritin, vitamin B12, folic acid, liver function test and hormone research. Centrifugation was performed for 10 minutes at 3000 rpm to separate the serum. All serum were kept at a deep-freezing temperature (-80 °C).

2.2 Control Group

The control group include 50 adult individuals (27 men and 23 women) from above 30 years age groups who with the normal RBC indices, B12 and folic acid levels, ferritin levels, liver function tests, and hormonal parameters were chosen.

2.3 Macrocytic Anemia

MA was identified in a total of 112 patients (70 men and 42 women). Normally, red blood cells (RBC) with an elevated MCV of >100f are considered to have macrocytosis (Cakmakli et al., 2019). Twelve one-hand diagnosed with had MA and MCV levels more than 95 fl). No individuals in the current investigation developed hypochromic MA (MCV 77 fl, Hb values 11.5 g/dl). Based on the levels of vitamin B12 and folic acid, the results of liver function tests, and hormonal parameters, the MA patients were separated into MMA and non-MMA groups.

2.4 Megaloblastic Anemia

The focus of laboratory examinations and discoveries regarding Megaloblastic anemia has laboratory features of vitamin B12 and folate insufficiency (Ayodele, 2018).

2.5 Non-Megaloblastic Macrocytic Anemia

Non-megaloblastic causes of macrocytic anemia. (Steensma, 2012) these causes are associated with increased MCV (Green and Mitra, 2017).

2.6 Complete blood count measurements (CBC)

The fully automated hematological analyzer assessed the following blood parameters: RBC counts, Hb, HCT, RBC indices (MCV, MCH, and MCHC), RDW, PLT count, platelet indices (PCT, MPV, and PDW), and total WBC count. (Boule and Kovacs, 2017)

2.7 Reticulocyte count

Miller's disc technique was used to count the reticulocytes, and the formula below was used to determine the proportion of reticulocytes in each rat:

$$\%Reticulocyte = \frac{TotalReticulocyt X}{\text{Total RBC}} 100\ \%$$

2.8 Reticulocyte count determination

Using an electronic analyzer Pentra 120, CBC and reticulocyte counts were calculated in EDTA whole-blood samples. (Horiba)

2.9 Determination of serum ferritin (SF)

The blood ferritin level was measured through using Universal testing e 411 immunoassay analysis (ECL-based; Roche Diagnostics, Germany) (Marzany et al.)

2.10 Thyroid hormone measurement

Have used ROCHE cobas E411 Rack automatic immunometry analyzer, total triiodothyronine (T3), thyroxin (T4), and thyroid-stimulating hormone (TSH) were measured, which is based on technology of ECL

2.11 Vitamin B12& Folic acid identification

The Cobas e 411 serological instrument (Roche Diagnostics, Germany) measured folic acid and vitamin B12 using ECL technology.

2.12 Analytical Statistics

Graph pad prism was utilized to perform the statistical study (version 90.2). Comparing the medians of various groups was done using one-way analysis of variance (ANOVA). P values of 0.05 or under were considered vital, as well as the data are shown as median - (interquartile ranges).

3. RESULT AND DISCUSSION

3.1 Diagnosis and differentiation of Macrocytic Anemia

According to the MCV collection of 112 samples collected for this study, 79 participants were MMA and 33 people who were non-MMA were omitted from the study. The age range for the 112 samples with MA was well above 30, and all of them had MA (MCV > 95 fl). Based on the results of testing for vitamin B12, folic acid, ferritin, liver function test, and hormonal variables, the MA patients were separated into MMA and non-MMA groups. The tests used in this study to distinguish MA revealed that a total of 112 participants had it, 33 instances had MMA, and 79 cases had non-MMA.

3.2 Hematological Parameters

3.2.1 Red blood cell indices

The comparison of the MMA, non-MMA and control groups, as well as the effects of MA on a variety of hematological parameters were among the outcomes of the current research as compared to the healthy person are shown in the table(1), table (2) and table (3), median of MCV, MCH, and RDW considerably increased ($p<0.001$), which demonstrated in the table (1) Result is in line with previous studies that found megaloblastic patients had significantly higher MCV, MCH, and RDW (Barani Velan, 2013, Rayes et al., 2020)and (Yoganathan, 2015) Another study (Bhuta, 2018) revealed that RBC and Hb levels were lower in hypothyroidism. they demonstrated that patients with thyroid dysfunction had lower MCH, with the majority of these patients having MCVs between 95 and 105 fl. This condition is known as macrocytosis causes RBCs to grow larger than normal due to insufficient RBC production, hence macrocytosis is measured in terms of MCV (Rajashekar et al., 2017). Patients with B12 and folate deficiency anemia have higher red cell distribution widths (Ahmed and Mohammed, 2020).

According to the current study, both MMA and non-MMA patients had highysignificantly lower ($p<0.0001$) Hb, HCT, and RBC counts than those in the control group. as shown in table (1) results agree with earlier research that is linked to severe reductions in RBC count, hemoglobin concentration, and HCT (Petrov et al., 2011). In addition, a North Indian investigation looked at the hematological characteristics of patients who were alcohol addicted (Quraishi et al., 2016) Particularly among individuals who drank more alcohol, the data demonstrated an increase in erythrocyte MCV, MCH, and a reduction in the mean value of RBC. Hematological parameters in male alcoholics were studied in another South Indian investigation (Jain et al., 2020). Moreover, this study found not significant change in MCHC between the MMA and non-MMA groups and the control group. macrocytic anemia include elevated

MCV readings (>100fl) and normal MCHC. (Jadhav and Khaparde, 2017). However, the median Retic.count in MMA significantly increased ($p<0.05$) noted in both megaloblastic and non-megaloblastic groups compared to healthy subjects. As evidenced by the results in table (1). This outcome is consistent with a different research (Aslinia et al., 2006). the peripheral blood reticulocyte maturation parameters may be useful in separating causes of macrocytosis caused by megaloblastic and non-megaloblastic cells (Chan et al., 2007). Rather than non-megaloblastic anemia, a high reticulocyte maturation value is more diagnostic of megaloblastic anemia (Yoganathan, 2015). Similarly, have demonstrated that reticulocytes can serve as an effective biomarker for differentiating B12 deficient anemia. (Balci et al., 2016).

Table 1: RBC count and indices in control, MMA and non-MMA patients Median (interquartile).

RBC count and indices	Groups		
	Control=50	MMA=33	Non-MMA=79
RBC(x106 /mm3)	4.460(4.198-4.845)	3.150(2.675-3.745)****	3.440(2.775-4.165)****
Hb (g/dL)	12.80(12.00-13.58)	10.80(9.500-12.05)****	11.00(9.750-12.75)****
HCT (%)	39.60(37.38-44.85)	32.20(27.95-40.45)***	35.75(29.78-43.18)***
MCH(pg)	29.25(28.13-30.60)	38.50(35.40-53.00)****	36.70(34.20-44.70)****
MCHC(g/dl)	33.70(32.58-34.75)	33.90(32.60-3520)	33.45(32.00-34.60)
MCV(fl)	87.30(84.73-90.30)	106.6(100.6-108.0)****	101.4(97.90-107.5)
DRW(%)	12.70(11.73-13.30)	14.60(13.75-17.70)***	14.50(12.35-17.10)***
R.COUNT	1.300(1.000-1.800)	2.700(1.480-2.700)*	1.800(1.800-2.710)***

Star (*) denoted the comparison between the control group with the Hash (#) denoted the comparison between the MMA with non-MMA

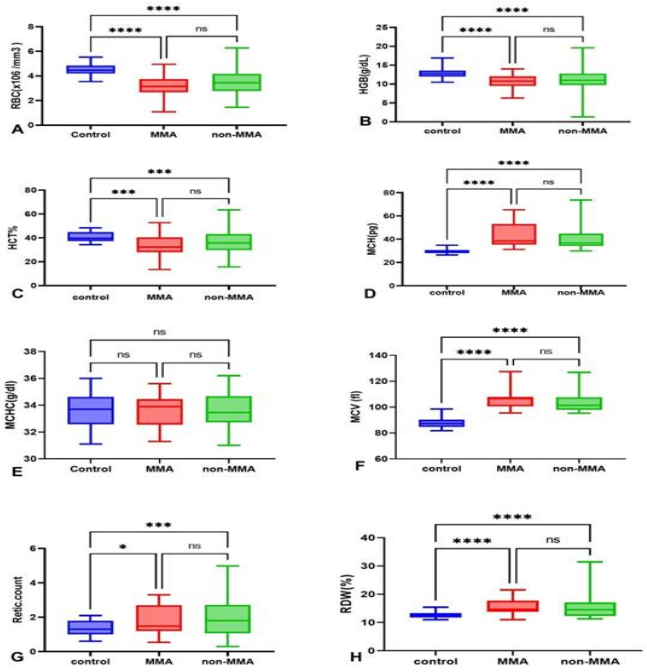

Figure 1: Box plot of (A) MCV, (B) Hb, (C) MCHC, (D) MCH, and (E) RDW and bar chart of (G) HCT and (F) RBC shows the comparison among control, MMA and non-MMA groups

3.1.2 Total and Differential Leukocyte Count

The results of the total and differential leukocyte count, as shown in table (2), and figure (2) showed that there were no observable changes between the groups of megaloblastic and non-megaloblastic patients in terms of total WBC count, granulocyte percentage, lymphocyte percentage, monocyte percentage, granulocyte percentage, and the absolute value of granulocyte and monocyte. As well as when the two groups were compared together. This is similar to the results of (Ransing et al., 2018); (Buke et al., 2020) and (Aktas et al., 2014) In this study, MMA and non-MMA participants had a significantly lower absolute value of lymphocytes than the control group. Similar observations were made by (Heerasing et al., 2016); (Rao, 2011).

According to(Sale et al., 2016) WBCs, particularly lymphocytes, can pass through the RBC channel's counting aperture and may thus be regarded as RBCs. The final RBC count is unchanged by the modest rise in RBC numbers. But because the analyzer bases its MCV calculation on the RBC histogram, lymphocytes that pass through the RBC channel inadvertently cause the MCV to rise. (Godon et al., 2012).

Table 2: WBC count in control, MMA and non- MMA patients (Median (interquartile ranges)).

WBC count	Control =50	MMA =33	NON MMA=79
WBC (x103 /mm3)	6.800(5.300-8.700)	6.100(3.300-9.350)	6.000(4.450-9.550)
LYM%	29.10(26.50-32.60)	29.55(24.10-35.50)	29.80(25.90 -36.00)
LYM(x103 /mm3)	2.300(2.000 -2.775)	2.000(1.500 -2.625)	2.000(1.5000 -2.425) *
MON%	6.100(5.600-7.400)	6.750 (5.425-7.175)	6.400(5.400 -7.400)
MON(x103 /mm3)	0.6000(0.4000-0.7000)	0.6000(0.4000-1.000)	0.6000(0.5000-1.100)
Gra %	60.90(55.25-63.60)	60.00(49.40-69.50)	60.60(48.60-65.50)
Gra (x103 /mm3)	4.250(3.350-4.700)	4.650(2.375 -7.150)	3.700(2.400-6.300)

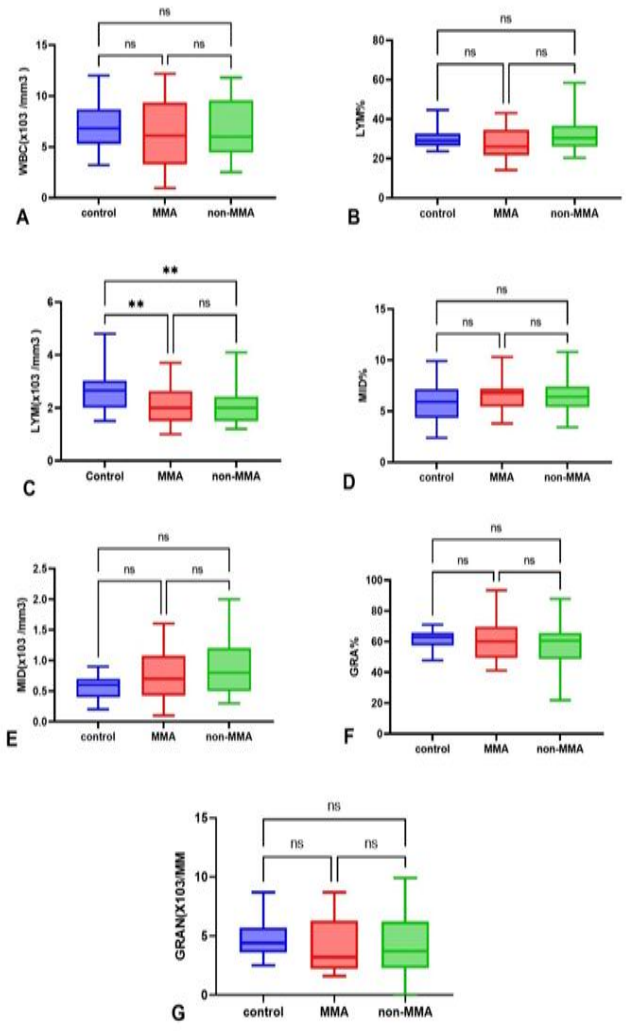

Figure 2: Box plot of (A) WBC count, (B) Granulocyte %, (C) Lymphocyte%, (D) Granulocyte, (E) Lymphocyte (F) Monocyte and (G) Monocyte % shows the comparison among control, MMA and non-MMA groups.

3.1.3 Platelet Count

According to the statistical analysis of the data. Both MMA and non-MMA participants' PLT very high significant decrease ($p<0.0001$) when in comparison to the normal subjects. As seen in table (3) and figure (3) This outcome is suitable with result of (Sharma et al., 2019). And also Table (3) showed that MPV significantly decrease ($p<0.0001$) in the non-megaloblastic patients when versus to the healthy subjects,. Additionally, the outcomes demonstrated that non- significant changes in PCT and PDW% in megaloblastic and non- megaloblastic with concerning to the healthy subject and also with each another. Low levels of platelets can occasionally be related to vitamin B12 or folate insufficiency, Erythropoiesis and thrombopoiesis require vitamin B12, Severe vitamin B12 deficiency may also result in thrombocytopenia in addition to anemia (Kansara and Sharma, 2022).

Table 3: Platelet count and indices in control, MMA and non MMA subjects (Median (interquartile ranges**)**.

PLT count	Control=50	MMA=33	Non-MMA=79
PLT (x103 /mm3	208.0(166.8-266.0)	127.0(88.00-184.5)****	173.0(109.0-220.0)**#
MPV(fl)	8.700(8.300-9.200)	7.800(7.200-8.700)****	7.850(7.175-8.800)****
PCT%	0.25.00(0.2100-23.80)	0.2900(0.2600-0.6575)	0.1800(0.1285-0.2675)
PDW%	13.70(11.05-15.18)	13.90(9.750-43.40)	13.50(11.20-39.70)

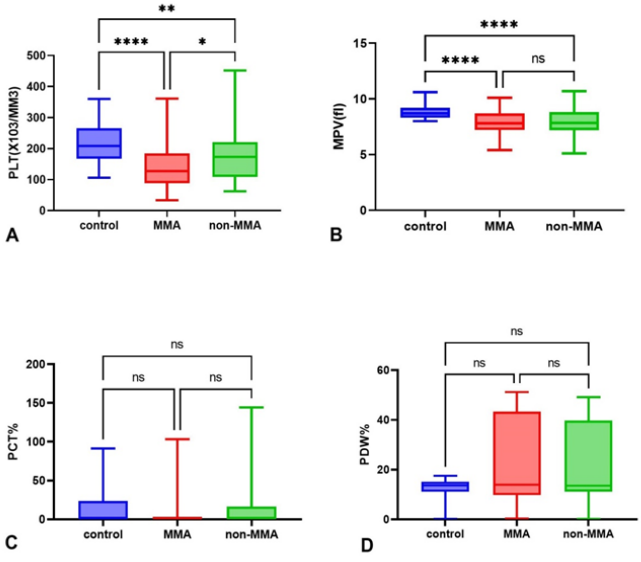

Figure 3: Box plot of (A) PLTcount and (B) MPV (fl) (C) PCT (D) PDW shows the comparison among control, MMA and non- MMA groups.

3.2 Thyroid function test

Table (4) and figure (4) indicates the consequence of MA on T3, T4, and TSH in MMA, non-MMA patient groups and healthy person. According to the current study, the results of the present study T3 a very high a significant (P<0.0001) decrease in non-MMA in comparison with that of healthy subjects and also significant decrease (p<0.05) in MMA. In addition to significant difference can be observed between both groups.as well as the statistical analysis show that high significant (p<0.001) decrease serumT4 in non-MMA, significant decrease (P<0.01) in MMA. This is in agreement with previous studies, which showed that patients had a significant decrease T4 (Mitrou et al., 2011) and (Aktaş, 2020).

Table (4) and figure (4) demonstrate that an extremely significant rise (P < 0.0001) of TSH in both group as contrast to the normal subject, as well as significant difference between MMA and non-MMA can be seen. This result is in line with other studies that revealed a significant increase in TSH in MMA patients (Aladily, 2022).

hypothyroidism is more frequently linked to anemia, and it can cause a variety of morphological anemias that can range in severity. Thyroid hormone shortage impacts not only Hb and total RBC count, but also several RBC indices such MCV, MCH, MCHC, and RDW. MCV values are higher in hypothyroid individuals compared to controls with normal thyroid function (Aparajita et al., 2022). Additionally, nutritional deficits of vitamin B12 and folate or malabsorption might enhance or contribute to which was before anemia in hypothyroid states (Patil, 2018).

Table 4: Serum T3, T4 and TSH in control, MMA and non-MMA Median (interquartile ranges)

Hormonal parameters	Group		
	Control=50	MMA=33	Non-MMA=79
T3	3.202(2.100-3.948)	2.410(1.683-5.348)*	2.210(0.9860-2.620)****#
T4	149.2(110.0-176)	123.9(94.81-162.7)**	103.5(61.79-145.9)***
TSH	1.900(1.300-2.800)	1.125(0.9265-2.505)****	2.730(2.035-3.725)***###

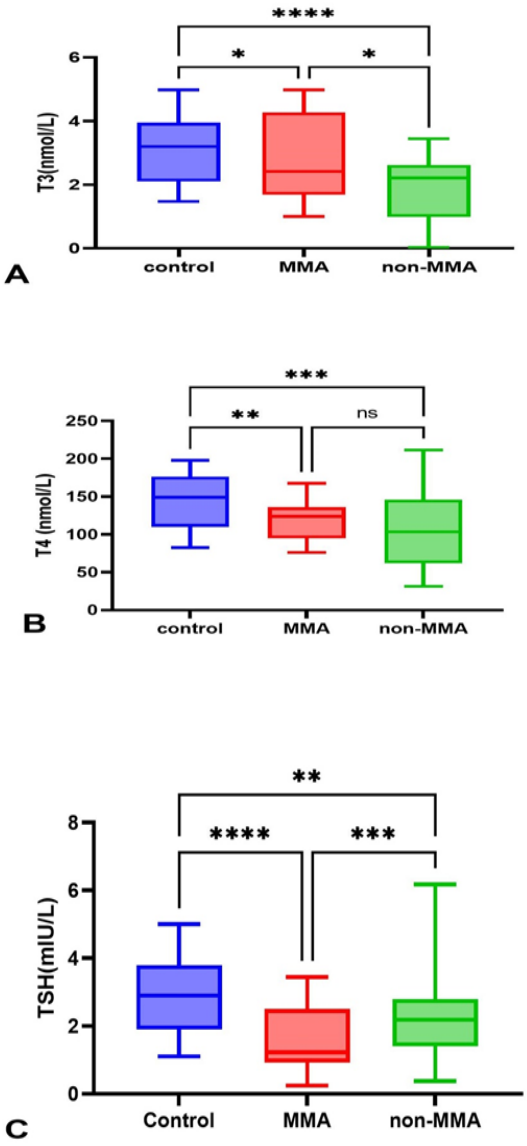

Figure 4: Box plot of serum (A) TSH and Bar charts of serum (B) T3 and (C) T4 shows the comparison among control, MMA and non-MMA groups.

3.4 Liver function tests parameter

Table (5) and figure (5) demonstrate that there was a very high significant (P<0.001) increase in median of serum AST in non-MMA and significant (p<0.01) increase in MMA were compared to the control group While there were non-significant difference between them. This study demonstrated that ALT was significantly increased in (P<0.0001) in non-MMA patients compared to control, and also stated that there was no significant change in ALT in MMA patients when compared with the healthy person. And also not significant difference between MMA and non-MMA groups. Its compatible with the study

of(Gómez-Medina et al., 2022) and (Abdel-Daim et al., 2016). another study who revealed that serum AST levels in non-MMA patients are significantly lower than those of the healthy individuals. the elevated erythropoietic activity is reflected by increased serum ALT levels (Yang et al., 2018)

The results also showed that there was a significant (P<0.0001) increase in median of serum TBIL in non-MMA on the other side significant (p<0.05) increase in MMA group in comparison with healthy person. It has been reported by previous study that serum TBIL level increased in MA patients (Rappai et al., 2019). No significant difference was observed in serum TBIL between MMA, non-MMA group to compared with one another. Macrocytosis associated with a megaloblastic marrow is usually accompanied by anemia due to ineffective erythropoiesis. The bone marrow is hypercellular, showing evidence of abnormal proliferation and maturation of multiple myeloid cell lines. These abnormalities are most evident in the erythroid precursors with large megaloblastic erythroblasts present in increased numbers throughout the marrow. This ineffective erythropoiesis is accompanied by intramedullary hemolysis causing elevated indirect bilirubin in the serum (Aslinia et al., 2006)

Furthermore ALP significant (p<0.01) increase in non-MMA as compared to the healthy person, and also ALP not significant change in MMA group when in comparison to the healthy subject. This result is in line with another study by (Fakhri et al., 2022) and (Kilo et al., 2016) Malnutrition and malabsorption leading to vitamin B12 and folic acid deficiencies resulting in macrocytosis and macrocytic anaemia have been acknowledged as cause especially in patients with alcoholic liver disease. (Scheiner et al., 2020)

Table 5: Liver function test (AST, ALT, TBIL, ALP) in control, MMA and non-MMA groups. Median (interquartile ranges)

Liver Function test	Groups		
	Control= 50	MMA=33	Non-MMA=79
AST	18.00(16.33 -26.75)	23.25(19.50-30.75)**	27.00(20.00-35.00)***
ALT	17.00(13.00-28.00)	16.00(13.75-24.25)	29.50(19.7538.50)*****####
TBIL	0.4000(0.1000-1.000)	0.7000(0.5000-1.050)*	0.9000(0.6000-1.225)****#
ALP	89.50(70.00-102.3)	88.00(75.00-113.5)	102.0(84.50-114.3)**

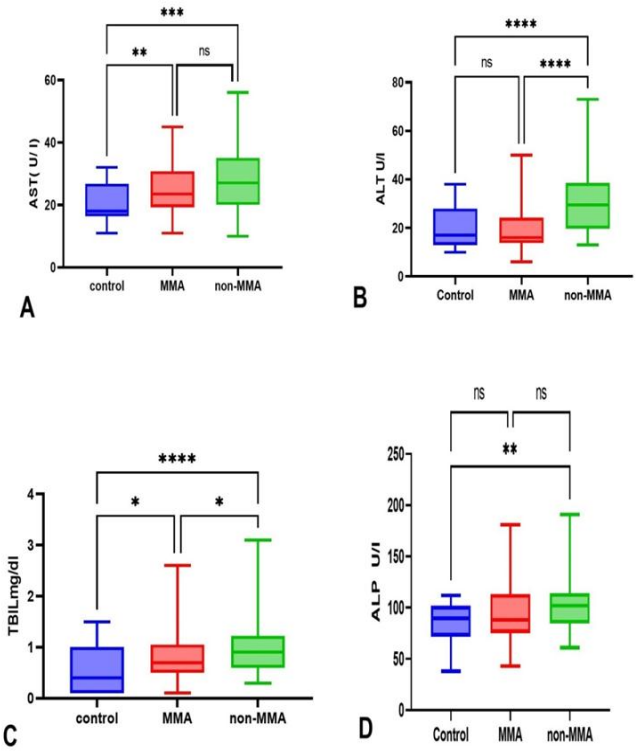

Figure 5: the Box plot of Liver function test (A) AST (B) ALT (C) TBIL (D) ALP

3.4 Biochemecial test (Ferritin ,Vitamin B12 and Folic acid)

As shown in table (6) and figure (6) there was extremely significant rise (P<0.001) in the median of serum ferritin in the non-MMA and (P<0.05) in MMA group as compared with control group. In contrast significant deference also detected between both of them. Our results supported by (Imashuku et al., 2016, Shalev et al., 2002) (Hsu et al., 2022) they Identifying that Macrocytic Anemia having a serum ferritin level more than 100 ng/mL. (Lanier et al., 2018)

The effects of MA on Folic acid as well as vitamin B12 in MMA, non-MMA patient groups, as well as healthy subjects were revealed in Table (4) outcomes from this study showed a significantly high decrease(p<0.0001) in serum B12 in MMA and non-MMA group (P<0.01) as compared with control group. In addition to significant deference between two groups. and this finding is compatible with the result of reference (Chapman et al., 2016, Abdulmanea et al., 2014) Furthermore, the statistical analysis of the results demonstrated that there was very high significant (p<0.001) in Folic acid in MMA group when compared with control group, despite the existence of significant change between MMA and non-MMA groups. This is in agreement with studies of(Chapman et al., 2016) and (Pandey, 2019)

Macrocytosis is the first abnormality can be seen in complete blood counts of patients with folate or vitamin B12 deficiency(Kendrick, 2010) Vitamin B12 and folate are the most important cofactors necessary for DNA synthesis. When they are deficient, it causes macrocytic change of RBC, abnormalities of leukocytes and platelets(Green, 2017)

First, vitamin B12 and folic acid deficiencies are the most likely cause of macrocytosis (MCV>100 fl), which leads to macrocytic anemia. Active B12 is commonly used as biomarker in the diagnosis of vitamin B12 deficiency(Heil et al., 2019) The present study evaluated the incidence of PA in patients with macrocytic anemia and in patients with low serum B12 (Abdulmanea et al., 2014).

Since macrocytosis is the earliest finding seen in patients with folate or vitamin B12 deficiency, in most cases, the classic expression of vitamin B12 deficiency is a macrocytic anemia with accompanying elevated MCV (Herrmann and Obeid, 2008).

Table 6: Serum Ferritin, B12 and Folic acid in control,MMA and non-MMA groups (Median (interquartileranges).

Biochemical test	Groups		
	Control=50	MMA=33	non MMA=79
Ferritin(ng/mL)	102.00(68.50-118.5)	215.6(88.65-509.5)***	273.4(153.1-499.1)****##
Vitamin B12	300.0(250.0-350.1)	114.4(65.15-150.4)****	295.2(215.4-376.7)####
Folic acid	10.00(8.000-15.00)	4.410(1.670-4.960)****	10.06(3.320-15.59)###

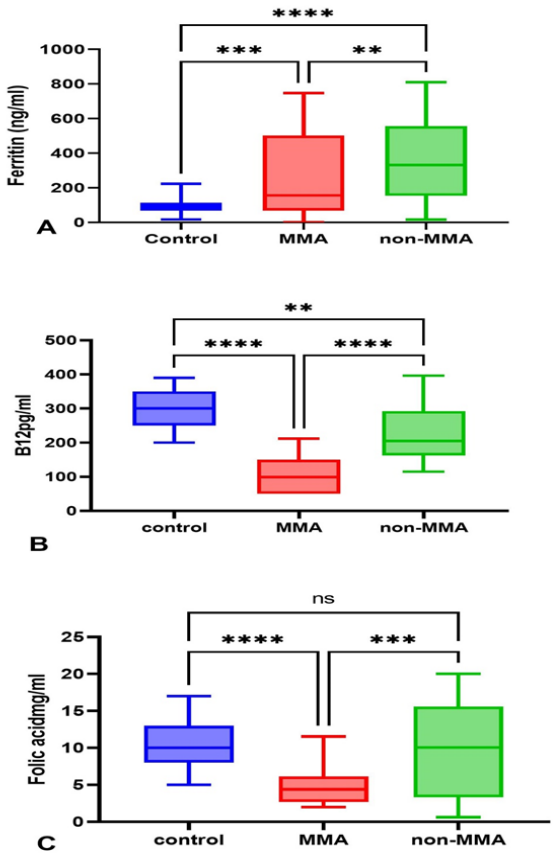

Figure 6: the box plot serum (A) Vitamin B12 (B) Folic acid in MMA and NON MMA

4. CONCLUSION

The outcomes of the study demonostrae that MCV, MCH, and RDW were increased, while RBC count, HB, HCT, and PLT count in MA patients were decreased. Furthermore, MCHC has non-significant changes. Thyroid hormones (T3)

and (T4) were decreased in both MMA and non-MMA, and serum (TSH) was increased inMMA and non-MMA. Futhermore, in liver function test TBIL highly significant increase in MMA and non-MMA, while significant increase AST in MMA and non-MMA and ALT significant increase in non-MMA, non-significant change MMA, While serum APL significant increaseinnonMMA,nonsignificantchangeMMA.Thebiochemicalparameters(SerumvitaminB12&Folicacid)arestrongbiomarkersforMMA.

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