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Research Article

Folic Acid Supplementation Role on Bone Marrow and Blood Cells Morphology during Methotrexate Treatment

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ABSTRACT
This study was aimed to investigate the effect of folic acid on the hematological picture and bone marrow of rats treated and none treated with methotrexate. A total of 40 adult rats were divided in to 4 groups, C-control group, F- received 0.07mg/kg B.wt folic acid daily, M-were received 0.03mg/kg B.wt Methotrexate three times a week.FM- received folic acid daily and methotrexate three times a week. After the end of the experiment which lasted for 9 weeks, blood and bone marrow samples were taken. The results of MTX group reveal abnormal RBCs shape. The bone marrow smears reveal a case of megaloblastic anemia characterized by mild erythroid hyperplasia. The myeloid: erythoide ratio is 8:1. The group of animals that received FA with MTX showed a good prognosis which characterized by high significant improvement of studied parameters. The myeloid: erythroid ratio was 3:1. Therefore, it was concluded that FA administration with Methotrexate corrected the deleterious effects of MTX on erythrocyte parameters and bone marrow.

Keywords: Anemia, Folic Acid, Methotrexate, Bone Marrow.

INTRODUCTION

Folic acid is a synthetic folate compound also known generically as folate or folacin, pteroylglutamic acid (PGA), is a member of the B-complex family of vitamins, and works in concert with vitamin B12.Folic acid functions primarily as a methyl-group donor in transferring one carbon atom involved in many important body processes, including building blockers of DNA and RNA needed for protein synthesis[2].Therefore, rapidly growing tissues, such as those of a fetus, and rapidly regenerating cells, like red blood cells have a high need for folic acid for erythropoiesis[3]. Methotrexate (MTX) and formerly known as amethopterin, is an antimetabolite and antifolate drug used in treatment of cancer and autoimmune diseases[4].It acts by inhibiting the metabolism of folic acid. Methotrexate replaced the more powerful and toxic antifolate aminopterin, and the two should not be confused with each other. Its mechanism of action is inhibiting the conversion of inactive folate [dihydrofolate (DHF)] to active folate [tetrahydrofolate (THF)][5]. Low-dose of MTX weekly is widely used in the treatment of rheumatoid arthritis and is now increasingly used in other rheumatic conditions, including systemic lupus erythematosus and juvenile arthritis. Because of its anti-metabolic and cytotoxic actions, questions of its safety in those of childbearing age are likely to become increasingly important[6]. Therefore, this study was designed to investigate the effect of folic acid on some erythrocyte parameters and bone marrow for female rats suffer from folic acid deficiency induced by MTX treatment.

MATERIALS & METHODS

Animals: A total of forty female Albino Wister rats were used in this study. Their age was 8-10 weeks. Their body weight ranged between 100-130 grams. All animals were kept in the same suitable environmental conditions of 25-27C^0, and photoperiod of 12 hours daily. The animals were housed in plastic cages of 50×35×15 cm in diameter. These cages were cleaned once a week. The food (pellets) and water (tap water) was given freely. The animals kept at least 2 weeks for acclimatization before beginning the experiment.

Experimental Design: Rats were divided into 4 groups equally as follows:-

1. Control (C) group: Ten rats were received distilled water.
2. Folic acid (F) group: Ten rats were received folic acid (0.07mg/kg body weight) daily [7].
3. Methotrexate (M) group: Ten rats were received methtrexate (0.03 mg/kg body weight) three times a week[8].

4. Folic acid and Methotrexate (FM) group: Ten rats were received folic acid (0.07 mg/kg body weight) daily and methotrexate (0.03mg/kg body weight) three times a week.

The drugs were given by intubation. The experiment was lasted for 9 weeks. At the end of the experiment, blood samples were collected andall animals were sacrificed for bone marrow examination.

2.3.Blood Collection: The animals were anesthetized by intramuscular injection of ketamine (90mg/kg B.W) and Xylazine (40mg/kg B.W). Blood samples were obtained via cardiac puncture technique from each anesthetized animal using 5 ml disposable syringe with needles 22G. Some of the samples were collected by disposable syringes after washing them with heparin and the blood kept in EDTA tubes. The other samples were centrifuged at 2500 round / minutes (rpm) for 15 minute and then a serum sample was stored in freezer at -18C° until they were used.

Parameters of the experiment: Blood film on a slid was made and left to dry in the air then stained the film with lieshman stain and smears were examined under oil emmersion. On the other hand, two drops of methylene blue stain was drawn in small test tube then 4 drops of fresh blood was added and mixed well. These tubes were left in the incubater at 37 Cºf or 15 minutes. After that the smears were prepared and dried by air and then examined under oil immersion lens[9].At the end of the experiment all animals were sacrificed after blood collection, the femur was broken after cleaning from the surrounding tissues; a special needle was introduced into it. Bone marrow smears were prepared by the two-slide technique as mentioned by [10] and stained with Giemsa stain.

2.5.Statistical analysis: One way ANOVA was performed to assess the significant differences among means[11]. Values were expressed as mean ± standard error.

RESULTS & DISCUSSION

Folic acid improvement on bone marrow in rats treated with Methotrexate.

1. Control Group

Myeloid/granulocytes series: There is a slight increase in the neutrophils metamyelocytes. Erythroid series: There is a slight decrease in the early stage of this pool with lack of nucleated RBCs, polychromatophilic cells and mature RBCs(figure1). Thrombocytes (platelets) series: Normal number and shape of megakaryocytes is seen. Myeloid: Erythroid= 4:1 which is very close to normal bone marrow smears[10].

2. Methotrexate Group

Figure (2) shows bone marrow section from rats treated with Methotrexate. Hyper cellular bone marrow of macrocytes with megaloblastoid rubricytes. Myeloid / granulocytes series: There is a moderate left shift in this series and moderate increase in the neutrophils metamyelocytes with some giant metamyelocyte and giant myelocyte with cells from earlier stages. There is a slight myeloid hypoplasia. Erythroid series: There is an increase in the early stage of this pool with lack of nucleated and mature RBCs. There is absence of iron storage. There is mild erythroid hyperplasia with left shift (increase of prorubricytes and basophilic rubricytes). Thrombocytes (platelets) series: Normal number with normal morphological appearance of megakaryocytes is seen. The myeloid hypoplasia (reduced granulopoiesis) due to defective nucleoprotein synthesis caused by folic acid deficiencies[12]. Myeloid: Erythroid= 1:8.The cytological findings of this group may be consistent with Megaloblastic anemia. This is mostly resulted from inhibition of DNA synthesis in RBCs. The most causes for such inhibition are folate deficiency that resulted from folic acid antagonists MTX which is immunosuppressive drug[13].This is resulted by the decrease in red blood cells (RBCs) count and hemoglobin concentration[14,15].

3. Folic Acid Group

The cells of the myeloid/granulocytes series are normal with a slight increase in number and have normal morphology. There might be a slight left shift. Erythroid series: within normal or there is a slight increase in the cells of the early stage of this pool with lack of nucleated RBCs, polychromatophilic cells and mature RBCs. There is absence of iron storage. Normal to mild erythroid hyperplasia (Figure3). Thrombocytes (platelets) series: within normal. These findings may be consistent with mildly active bone marrow characterized by increase production of WBC and RBCs. This is coincided with our previous results which shows increase RBCs number, Hb content and PCV% in this group[15]. Myeloid: Erythroid=3:1.

4. Folic Acid and Methotrexate Group

Myeloid/ granulocyte series.There is a mild increase in this pool with marked increase in the neutrophils metamyelocyte and there is a decrease in bands as well as mature neutrophils. At the meantime, there is a mild myeloid hyperplasia with left shift. This could be explained by an increase in the granulocytic precursor with absence of bands or mature neutrophils which is an indicative of maturation arrest at the level of metamyelocytes which is

usually seen with inflammation (figure 4). Erythroid series: There is a mild decrease in this pool with lack of RBCs, polychromatophilic and mature RBCs. There is absence of iron storage. At the same time there is a moderate erythroid hypoplasia with significance maturation arrest. This is most likely consistent with anemia of chronic disease, decrease iron, and folate or vitamin B12 deficiency[16]. Thrombocytes (platelets) series: There is a marked decrease in the megakaryocytes. No lymphocytes, plasma cells or their precursors can be found. Myeloid: Erythroid= 3:1.

Folic acid improvement in blood cells morphology in rats treated with Methotrexate.
Blood smear from rats treated with Methotrexate shows megaloblastic changes characterized by oval, macrocytic (large than normal size) red cells, polychromatophilic, marked anisocytosis (increased variation in RBCs sizes) and poiklocytosis (abnormal shaped RBCs) with hyper segmented neutrophiles containing Dohles' bodies (large gray bluish inclusion bodies near the peripheral of the cytoplasm). Anisocytosis, Poikilocytosis and macrocytes are present in the blood smear of MTX group but not in control and folic acid groups. All these shapes of RBCs are shown in blood film of this group (figure B). These characteristics, changes are in agreement with other studies in rats suggested a case of megaloblastic anemia in this group [17].

CONCLUSION

It could be concluded that folic acid supplementation cause an increase in the synthesis and maturation of highly resistance RBCs. Bone marrow examination reveals that its supplementation with MTX treatment produce 3:1 myeloid: erythroid ratio instead of 8:1 in MTX group which indicates megaloblastic anemia.

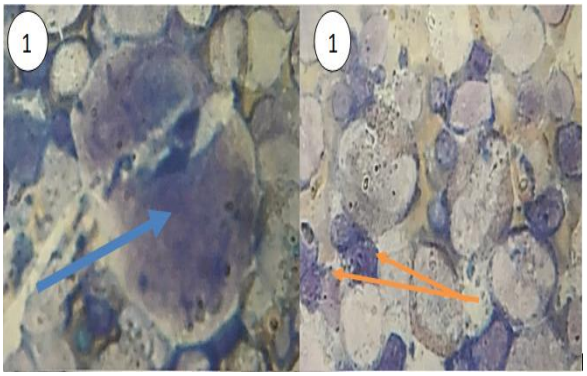


Fig.1: Light microscopic photograph of Bone marrow for control group showed large cell of polychromatophilic rubricyte in various stages of hemoglobin synthesis (Giemsa×1000).

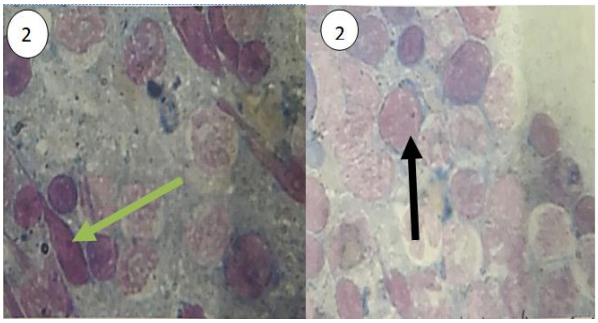


Fig.2: Light microscopic photograph of Bone marrow for Methotrexate group showed Megaloblastic erythroid in an aspirate smear and macrophilic polychromatophilic rubricyte at the top as well as nutrophilic hyperplasia (Giemsa ×1000).

= Mature megakaryocyte
= Eosinophilic megakaryocyte
= Neutrophilic hyperplasia
= Megakaryoblastic rubricyte

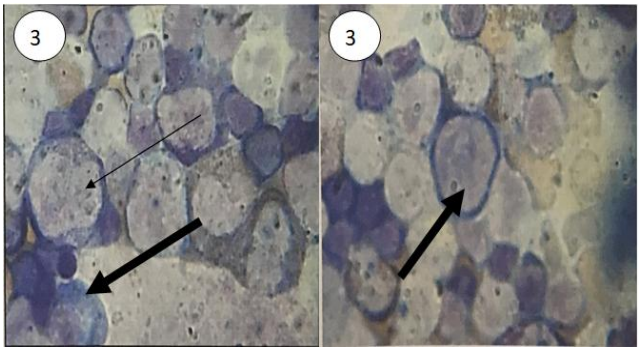


Fig.3: Light microscopic photograph of Bone marrow for folic acid group showed polychromatophilic rubricyte in various stages of hemoglobin synthesis of myeloblast (Giemsa ×1000).

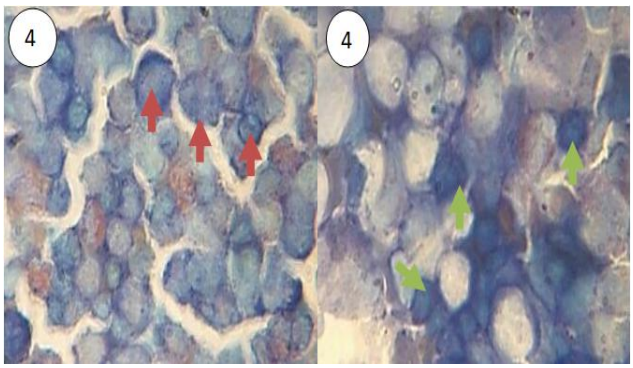


Fig.4: Light microscopic photograph of Bone marrow for folic acid and Methotrexate group showed erythroid hyperplasia with increase number polychromatophilic erythrocytes (Giemsa ×1000).

= Polychromatophilic rubricyte
= Polychromatophilic erythrocyte
= Erythroid hyperplasia

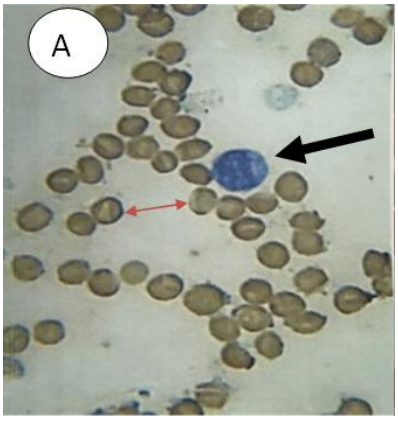


Fig.5A: Light microscopic photograph of blood smear for control group showed normal shape and size with normal reticulocyte and lymphocyte in blood (Leishmans×100).

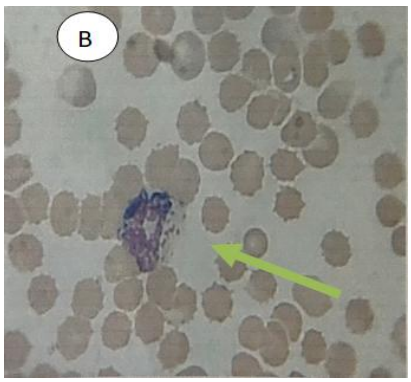


Fig.5B: Light microscopic photograph of blood smear from Methotrexate group showed toxic band neutrophile with foamy basophilic cytoplasmic granules (Dohel bodies) in peripheral blood (Leishmans ×100).

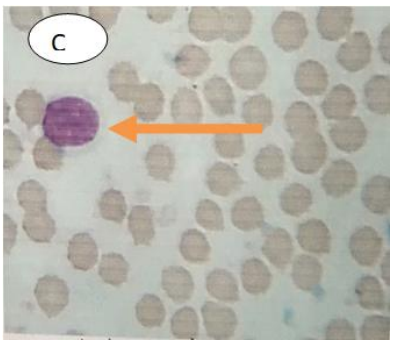


Fig.5C: Light microscopic photograph of blood smear form Folic acid group showed normal RBCs in size and shape in peripheral blood (Leishmans ×100).

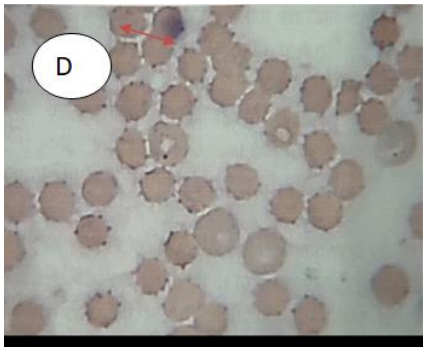
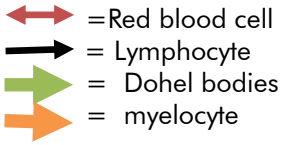


Fig.5D: Light microscopic photograph of blood smear form Methotrexate and Folic acid group showed gaint toxic normocytic normochromic in peripheral blood (Leishmans ×100).



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