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Histological effect of iron supplementation on bone marrow and peripheral blood cells of rabbits

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ABSTRACT

The aim of this study is to evaluate iron deficiency and iron overload on the structure of the bone marrow as well as the peripheral blood cells of rabbits *(Oryctolagus cunicuolus)* as a model of experimental animals. For this purpose twenty four premature rabbits were divided into four groups, two control groups, sex-well fed animals and sex starved animals, and two experimental and supplemented groups, sex-well fed animals and sex starved animals were daily supplemented with therapeutic dose of iron as Fumarate tablets. Smears from bone marrow and peripheral blood were prepared. The bone marrow of the starved without iron supplementation showed normal appearance. However, the stem cell progenitors were predominantly in the last stage of development the reticulocytes were appeared with clear cytoplasm. By continuous iron supplementation the bone marrow cells restored their normal distribution and shapes. By time stainable materials were deposited within the cytoplasm and nuclei of the pro- erythroblasts. The reticulocytes become irregular in shape. These abnormalities took place in the red blood cells. In the well fed and iron supplemented groups the stainable materials were badly accumulated in the bone marrow cells from the first week of administration. These results concluded the severe need of iron to treats iron deficiency in a limited time. However, long duration of iron supplementation will introduce certain types of abnormalities in bone marrow and peripheral blood cells.

Key words: Histological effect, bone marrow, peripheral, blood cells, rabbits.

INTRODUCTION

Functional iron deficiency was defined as a pathological state in which the bone marrow erythropietic capacity to response to erythropoietin (epoetin) is limited by iron release from storage depots. The result is utilization of higher and more costly doses of epoetin to overcome what is errantly perceived as relative resistance to epoetin (Besarab *et al.,* 1999) . The inability to absorb iron is quantity sufficient to match the demands of heightened erythropoiesis constitutes, the mechanism of iron deficienry. This may occur even when 200 mg of elemental iron is ingested. Iron absorption varies inversely with ferritin levels (Eschbach *et al.,* 1977) , ferritin levels exceeding 100 ng/dl do not guarantee adequate marrow iron storage delivery. Intestinal iron absorption is decreased by gastric proton pump inhibitors and H2 - antagonists; by ingestion of dietary phytates, oxalates, carbonates, phosphate and tannates (Conrad, 1987); and by calcium containing phosphorus- binding compounds that blocked iron uptake by entrocytes (Whiting, 1995). Finally iron replete individuals manifest decreased iron stores with three months of protein treatment (Markowitz *et al.,* 1997) .

The most direct assays quantify changes in polyunsaturated fatty acids or advanced oxidation of protein products as the potentially noxious effects of increased iron burden and free radical induction of iron (Peuchant *et al.,* 1994 and Epperlein *et al.,* 1998). Changes in antioxidant enzyme systems, including catalase , glutathione peroxidase and supra oxiddismutase are also used to interfere the *de novo* generation of reactive oxygen species. During the periods of oxidative stress, and increase bodily iron content may represent increased liability

for the ill effect. Increased tissue iron content amplifies free-radical mediate oxidative tissue damages (Van der Kraaij *et al.,* 1988, and Delmas - Beauvieux *et al.,* 1995).

Ferric ion is bound to mucin in stomach and delivered to duodenum, where it can be absorbed. Iron is transported across the apical membranes of the gut mucosa by integrin. Once within the mucosal cells, iron may be stored, utilized in protein synthesis, or exported to the serum where it is carried by transferrin. Differed transferrin bind to the transferrin receptors on the surface of the cells and is endocytosid. In the cells, iron is bound to low and high molecular weight ligant and is thought to shuttle iron with the cells. Iron can be stored intracellularly within ferritin, or can be utilized by mitochondria including aconitase and cytochromes (Lash and Saleem, 1995).

The initial tissue localization and redistribution of radioactive iron injected intravenously into the rat as ferritin, chondroitin sulfate and non-viable red cells was determined. Ferritin iron initially localized in the hepatocyte showed minimal redistribution of 24 hrs., in normal animals. All forms of iron were actively mobilized in iron deficient animals (Kim *et al.,* 1985). Iron was accumulated in spleen macrophages ferroportin and ferritin expression its content in the spleen decreased while a progressive storage of iron occurred within hepatocytes which were paralleled by a significant increase hepcidin. Under this condition, iron was still absorbed from duodenal lumen as divalent metal and incorporated into duodenal ferritin and iron transfer to the circulation was reduced (Theurl *et al.,* 2005).

Functional iron deficiency develops during treatment with recombinant human erythropoietin and in the infectious state and during the inflammatory process. This accelerates erythropoesis and by so increases iron requirement frequently for higher than the ability of iron stores to transfer iron into bone marrow (Prusak and Grzegorzewska, 2002). An inadequate iron supply to bone marrow and the rate at which iron can be drowning existing store may easily limited the rate of delivery of hemoglobin synthesis. This may result in functional iron deficiency (Schaefer and Bahner, 2000).

Malnutrition owing to inappropriate dietary intake and a high prevalence of iron deficiency anemia has been reported (Wadhwa *et al.,* 1997). This anemia caused by low iron- folic intake and complicated by alcohol consumption (Coetzee *et al.,* 1994) . The aim of this study is to test of both iron deficiency and iron over load on the structure of developed bone marrow cells as well as the peripheral blood cells of rabbits as a model experimental animal.

MATERIAL AND METHODS

Premature rabbits with mean weight of 0.75 kg were used. They were brought from a private farm in Jeddah, Saudi Arabia. The animals were acclimatized for one-week before experimentation, during which they were fed commercial diets, vegetables crushed wheat and corn as a complete diet. They were housed in stainless steel cages under room temperature and air conditioning. Then they divided into four groups: Two controls (6 each); one well fed by the mentioned food and the other severely starved and two experimental groups (9 each) one well fed and the other starved. The starved groups were fed small amount of crush wheat and corn ones every morning (low iron diet). The experimented groups were supplemented daily by iron in dose equivalent to the human therapies (as Fumarate tablets).

24 Rabbit

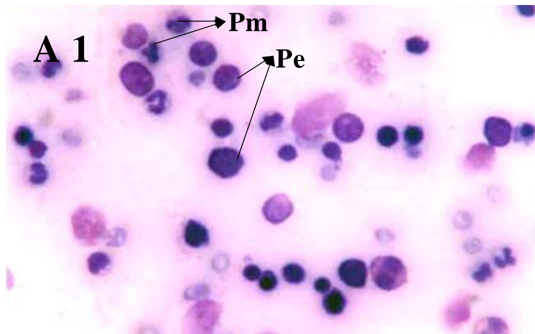
Control groups		**Experimental groups**	
Well-fed (6 Rabbits)	Starved (6Rabbits)	Well fed (6Rabbits)	Starved (6 Rabbits)
No iron supplementation		Daily iron supplementation	

Iron fumarate was dissolved in distilled water to achieve the therapeutic dose of human 66 mg / 70 Kg person and calculated according to the body weight of rabbits (0.7 mg iron/ rabbit) in one ml of distilled water the doses were forced - fed by stomach tube daily till 21 days. After each week one animal was randomly selected from each of the four groups and sacrificed. Fresh blood was smeared, methylated fixation and stained by Giemsa and Lieshmann stains. Bone marrow was collected from the head of long bones, smeared, and stained as mentioned on fresh blood.

RESULTS

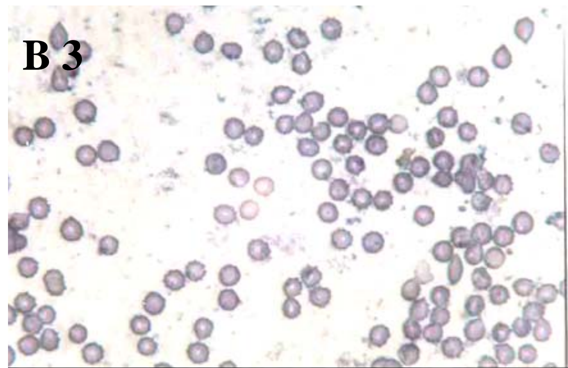
Microscopic Examination

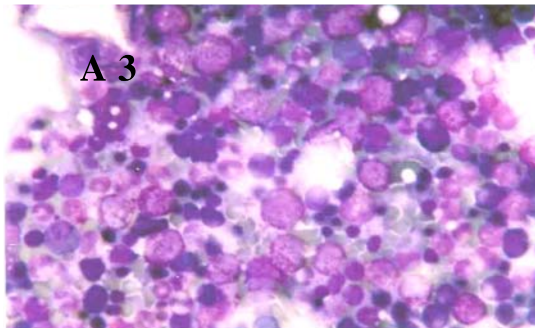
Blood and bone marrow smears of the four groups, controls (well fed and starved) and experimental (well fed and starved were observed microscopically:



Starved groups:

After one week the blood and bone marrow smears appeared normal in iron-unsupplemented group. However, the stem cells progenitors were predominantly in the last stage of development. The reticulocytes with pale and empty cytoplasm were also found. In the starved and iron - supplemented group, the bone marrow reticalocytes appeared to have irregular cell membrane after one week of administration. By time the stem cells progenitors restored their normal shapes and distribution. However certain accumulation of stainable materials were found in proerythro blasts which increased in time dependent manner.





Well fed groups

After one week of observation the bone marrow of iron supplemented and well fed group looked to be dark stained smears. This dark stains scattered among proerythroblasts and megakaryoblasts while the promyeloblasts appeared normal. These dark stained progenitors yield dark stained reticulocytes and erythrocytes with normal cytoplasm and cell membrane,. Finally the erythrocytes and the bone marrow cells of iron - supplemented and well fed group showed severe accumulation of dark granules within the cytoplasm and nuclei of the proerythroblast. In the opposite side the unsupplemented and well-fed group showed any of these alterations (Figs. A: 1, 2, 3, 4; B: 1, 2, 3).

Fig. (A), 1

Normal bone marrow showing different types of stem cell progenitors, proerythroblasts (pe) and promyeloblasts (pm), X 1000, **2:** treated bone marrow showing absence of promyeloblasts and presence of megakaryoblast (me) X 1000, **3:** treated bone marrow showing eosinophil- proerythroblasts and absence of basiphil-proerythroblasts and promyeloblasts. X 1000, **4:** treated bone marrow showing stainable granules in the cytoblasm and the nuclei of the proerythroblasts X 1000 (Giemsa and Lieshman stains).

Fig. (B), 1

Treated blood smear showing hypochromic anemia due to severe starvation X 440, **2:** treated blood smear showing abnormal shaped erythrocytes and accumulation of stainable materials inside the erythrocytes X 440, **3:** treated blood smear showing fine stains within the cytoblasm and barr shape erythroblasts X 440 (Giemsa and Lieshman stains).

DISCUSSION

Bone marrow precursor cells play a critical role in bone marrow maintenance and regeneration. Morphological finding of standardized features, cellularity, megakaryoblastes, eosino- pills, cellular debris, plasmocytosis and iron laden cells are a significantly diagnostic validity (Thiele *at al.,* 2005). Any disturbance caused by nutrient supplementation, or by exogenous substances will be followed by abnormal morphological findings of these cells. Severe starvation and continuous iron administration or shorten of the nutrient or are considered potential effective at high level. So the severely starved animals without iron supplementation showed certain types of anemia, specifically hypochromic anemia (iron deficiency). Thomas and Thomas (2002) evaluated biochemical markers to distinguish iron deficiency from anemia of chronic disease and determined the increase of reticulocyte and low hemoglobin content because these cells are the eartiest cells released into the blood and circulate for only 1 to 2 days (Mast *et al.,* 2002). Iron supplementation is successfully combated the anemia developed - to some extent - in these severely starved animals for a short time. This is may be due to the short duration of iron administration or to the malabsorption of iron doses. This observation was also found by (Fudin *et al.,* 1998), who introduced an example that patients with no stainable marrow in creased their hemoglobin levels to normal by iron succharate therapy at a weekly dose of 62.5 mg.

In the present study hemoglobin content raised, but not exceeded the control values in starved groups after one week of daily iron administration. Many evidences reported that iron supplementation failing to restore hemoglobin concentration to normal (Beaton and Mc Cabe, 1999), common reasons are that inadequate amount of iron due to the problems such as low compliance, malabsorption, short duration or high iron requirement during such periods (Hallberg, 1994) .

Iron parameters like serum ferritin, iron saturation and serum iron are routinely used in diagnosing of iron deficiency, and they may be influenced by many factors. Our results showed a decreased of serum iron than normal in starved animals without supplementation and increased nearby control in starved and iron supplemented group after the first week of observation However, serum iron highly increased than normal in both starved and supplement and well fed and supplemented groups. Ong *et al.,* (2005) showed that low serum ferritin followed by serum iron < 60 mg / dl < 7 % (respectively) in iron deficient patient, and the absence of stainable iron in the bone marrow was also diagnostic of iron deficiency (Hanif *et al.,* 2005) .

In a study of level of serum transferring receptor in children with different stages of iron deficiency Lin *et al.,* (2001) reported a significant improve to iron deficiency anemia after iron supplementation. To understand iron nutritional status in incidence of sub clinical iron deficiency and effect of iron supplementation Lin *et al.,* (2003) concluded that iron supplementation was important for patients to prevent the occurrence of iron deficiency anemia. Theurl *et al.* (2005) concluded that iron overload results in iron accumulation in certain cells *via* macrophages and latten in hepatocytes in parallel to transfer of iron from gut to circulation, thus presenting body iron accumulation. In macrophages the ferroportin has been shown in the intercellular vesicles (Delaby *el al.,* 2005). This study, showed an accumulation of iron as cytoplasmic and nuclei granules. The most obvious observation was the restoring of stem cell progenitors to their normal appearance. However, certain alterations in the cell membrane and some accumulation of stainable material in the cells of the starved and iron supplemented groups. These alterations severely increased in the well-fed iron supplemented groups, which reflected iron over load of these animals. Erythroidal cells from patients with anemia analyzed for the distribution of cytoplasmic ferritin and mitochondrial ferritin. About 1/4 one fourth of normal proerythrobast showed diffused cytoplasmic ferritin (Cazzzola *et al.,* 2003) . Early erythroblasts from patient with anemia showed constitutive mitochondrial release of cytochrom C and a display aberrant accumulation of mitochondrial ferritin. This occurred at a very early stage of eythroidal differentiation (Tehranchi *et al.,* 2005).

Cereal and legume based diets contain low amount of bioavailable iron not only increase the risk of iron deficiency but also is a cause of iron deficiency anemia (Zimmermann *et al.,* 2005). However, a study on iron deficiency anemia indicated that lack of protein, calcium, Vitamins, A, B1, B2 was more serious. The balanced diet beneficial to hemoglobin, free erythrocyte prophyrin, serum ferritin content and improved the biological activities (Cai and Yan, 1990). Iron deficiency anemia associated with certain disease and continued for a longer time even after iron supplementation. The early detection and treatment and prophylactic iron folic acid supplementation would go a long way to optimize the biological activities (Kapur *et al.,* 2003).

REFERENCES

1. Beaton, GH. and McCabe, GP. Efficacy of intermittent iron supplementation in the control of iron deficiency anemia in developing contries. *The micronutrient initiative,* Ottawa, Canada (1999).
2. Besarab, A., Frinak, S. and Yee, J. Reviews: An indistinct balance, the safety and efficacy of parenternal iron therapy. *J. Am. Soc. Nephrol,* **10**: 2029 - 2043 (1999).
3. Cai, MQ., Yan, WY. Study on iron nutritional status in adolescence. *Biomed. Environ. Sci.,* **3**: 113 - 119 (1990).
4. Cazzola, M., Invernizzi, R., Bergamashi, G., Levi, S., Corsi, B., Travaglino, E., Rolandi, V., Biasiotto, G., Drysdale, J. and Arosio, P. Mitochondrial ferritin expression in erythroidal cells from patients with sideroblastic anemia. *Blood,* **101**(5): 1996 - 2000 (2003).
5. Coetzee, MJ., Badenhorst, PN., de Wet, JI., and Joubert, G. Hemotological condition of the San (Bushmen) relocated from Namibia to South Africa. *S. Afr. Med. J.* **84**: 416-420 (1994).
6. Conrad, M. Iron absorption. In: Physiology of the Gastrointestinal Tract, edited by Johnson L, New York, Raven, 1437-1453 (1987).
7. Delaby, C., Pilard, N., Goncalves, AS., Beaumont, C. and Canonne- Hergaux, F. The presence of iron exporter ferroportin at the plasma membrane of macrophages is inhanced by iron loading and downregulated by hepcidin. *Exp. Cell Res.* **310**: 43-53 (2005).
8. Delmas-Beauvieux, MC., Combe, C. and Peuchant, E. Evaluation of red blood cells lipoperoxydation in hemodialysed patients during erythropoietin therapy supplemented or not with iron. *Nephron,* **69**: 404-410 (1995).
9. Epperlein MM., Nourooz - Zadeh, H., Jayasena, SD.; Hothersall, JS., Noronha - Dutra, A. and Neild, GH. Nature and biological

significance of free radicals generated during bicarbonate hemodialysis. *J. Am. Soc. Nephrol,* **9**: 457-463 (1998).

10. Eschbach, J.W., Cook, JD., Scribner, BH. And Finch, CA. Iron balance in hemodialysis patients. *Ann. Intern. Med.,* **87**: 710-713 (1977).
11. Fudin, R., Jaichenko, J., Shostak, A., Bennett, M. and Gotloib, L. Correction of uremic iron deficiency anemia in hemodialyzed patients : A prospective study. *Nephron,* **79**: 299-305 (1998).
12. Hallberg, L. Prevention of iron deficiency . *Baillieres Clin. Hematol.,* **7**: 805-814 (1994).
13. Hanif, E., Ayyub, M., Anwar, M., Ali, W. and Bashir, M. Evaluation of serum transferrin receptor concentration in diagnosing and differentiating iron deciency anemia from anemia of chronic disorder. *J. Pak. Med. Assoc.,* **55** (1): 13-16 (2005).
14. Kapur, G., Patwari, AK., Narayan, S., and Anand, VK. Iron supplementation in children with celiac disease. *Indian J. Pediatr.,* **70**: 955-958 (2003).
15. Kim, BK., Huebers, H., Pippard, MJ. and Finch, CA., Storage iron exchange in the rat as affected by deferoxamine. *J. Lab. Clin. Med.,* **105**(4): 440-448 (1985).
16. Lash, A. and Saleem, A. Iron metabolism and its regulation. A review: *Ann. Clin. Lab. Sci.,* **25**(1): 20-30 (1995).
17. Lin, X., Lont, Z., and Shen, X. Changes of serum transferring receptor in children with iron deficiency and its response to iron supplementation. *Zhonghua Yu Fang Yi Xue Za Zhi.*, **35**(5): 325-328 (2001).
18. Lin, XM., Wang, Z., Shen, XY., Liu, WJ., Guo, YM., and Tang, Y. Iron status and affect of early iron supplementation on sub clinical iron deficiency in rural school - age children from mountainous areas of Beijing. Zhonghua Yu fang Y: *Xue za Zhi.,* **37**: 115-118 (2003).
19. Markowitz, GS., Kahn, GA., Feingold, RE.; Coco, M., and Lynn, RI. An evaluation of the effectiveness of oral iron therapy in hemodialysis patients receiving recombinant human erythropoietin. *Clin. Nephrol.*, **48**: 34 - 40 (1997).
20. Mast, AE., Blinder, MA., Lu, Q., Flax, S. and Dietzen, DJ. Clinical utility of the reticulocyte hemoglobin content in the diagnosis of iron deficiency. *Blood,* **99**(4): 1489-1491(2002).
21. Ong, KH., Tan, HL., Lai, HC. And Kuperan, P. Accuracy of various iron parameters in the prediction of iron deficiency in an acute care hospital. *Ann. Acad. Med. Singapore,* **34**(7): 437-440 (2005).
22. Peuchant, E., Carbonneau, MA. And Dubourg, I. Lipoperoxidotion in plasma and red blood cells of patients undergoing hemodialysis : Vitamins A, E, and iron status. *Free Radical Biol. Med.,* **16**: 339-346 (1994).
23. Prusak, M. and Grzegorzewska, AE. Causes of disturbance in iron turnover in chronic renal failure. *Pol. Merkuriusz lek.,* **12**(70): 309-313 (2002).
24. Schaefer, RM. And Bahner, U. Iron metabolism in rh EPO treated hemodialysis patients. *Clin. Nephrol.*, **53**: 565-568 (2000).
25. Tehranchi, R., Invernizzi, R., Grandien, A., Zhivotovsky, B., Fadeel, B., Forsblom, AM., Travaglino, E., Samuelsson, J., Hast, R., Nilsson, L., Cazzola, M., Wibom, P. and Hellstromlindberg , E. Aberrant mitochondrial iron distribution and maturation arrest characterize early erythroidal precursors in low-risk myelod splasmic syndromes. *Blood,* **106**(1): 247-253 (2005).
26. Theurl., I., Ludwiezek, S., Eller, P., Scifert, M., Artner, E., Brunner, P. and Weiss, G. Pathways for the regulation of body iron homeostasis in response to experimental iron over load. *J. Hepatol.,* **43**: 711-719 (2005).
27. Thiele, J., Kvasnicka, HM. and Dichl, V. Bone marrow features of diagnostic impact in erythrocytosis. *Ann. Hematol.,* **84**(6): 362-367 (2005).
28. Thomas, C. and Thomas, L. Biochemical markers and hematological indices in the diagnosis of functional iron deficiency. *Clin. Chem.,* **48**: 1066 - 1076 (2002).
29. Van der Kraaij, AM., Mostert, LJ., van Eijk, HG. and Koster, JF. Iron - load increases the susceptibility of rat to oxygen reperfusion damage : Protection by antioxidant (+) Cyanidanol - 3 and deferoxamine. *Circulation,* **78**: 442-449 (1988).
30. Wadhwa, A., Sabharwal, M., and Sharma, S. Nutritional status of the elderyly. *Indian J. Med. Res.,* **106**: 340 - 348 (1997).
31. Whiting, SJ. The inhibitory effect of dietary calcium on iron bioavailability: A cause for concern? *Nutr. Rev.,* **53**: 77-80 (1995).
32. Zimmermann, MB., Chaouki, N., and Hurrell, RF. : Iron deficiency due to consumption of a habitual diet low in bio available iron : a longitudinal cohort study in Moroccan chidren. *Am. J. Clin. Nutr.*, **81**: 115-121(2005).