EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 3
Issue: 10
22-25
Year: 2016

ASSESSING DIETARY PATTERN V/S REFRACTORY ANEMIA ON BONE MARROW FINDINGS

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Article Received on 28/07/2016 **Article Revised on 17/08/2016** **Article Accepted on 06/09/2016**

ABSTRACT
World Health Organization (WHO) defined Anemia as a condition in which the number of red blood cells or their oxygen carrying capacity is insufficient to meet Physiological needs, which vary by age, sex, altitude, smoking and pregnancy status. Patients present with the complaint of Refractory Anemia (RA). Refractory Anemia is a clonal disorder originating from a totipotent stem cell or from a multipotent myeloid progenitor cell, characterized by ineffective hemopoiesis and dyserythropoiesis. It is part of the heterogeneous category of Myelodysplastic syndrome. The aetiology of megaloblastic anaemia is diverse but a common basis is impaired De-oxy Ribonucleic Acid (DNA) synthesis. Most common causes of megaloblastosis are vitamin B12 and folate deficiencies. Cobalamin metabolism and folate metabolism are intricately related, and abnormalities in these pathways may lead to the attenuated production of DNA. So, there is increased worldwide concern about the consequences of folic acid and vitamin B12 deficiencies on health, which includes megaloblastic anemia. Iron deficiency is also an important and most common cause of Iron deficiency anemia worldwide. In developed countries, the prevalence of Iron deficiency anemia has decreased rapidly during the past few decades, but in developing countries like India it is still an important cause. So, for patients presenting with Refractory Anemia on initial work up bone marrow (BM) aspirate and bone marrow biopsy were done which was further correlated with dietary habits.

KEYWORDS: Refractory anemia, Megaloblastic anaemia, Iron deficiency anemia, Bone marrow aspiration.

INTRODUCTION
Bone marrow is the oldest surgical practice that continues to have clinical relevance in modern times. A wide variety of clinical conditions, primarily or secondarily affecting bone-marrow may present with Anemia. RA is a clonal disorder originating from a totipotent stem cell or from a multipotent myeloid progenitor cell, characterized by ineffective hemopoiesis and diserythropoiesis. It is unresponsive to treatment. It is part of the heterogeneous category of myelodysplastic syndrome. RA usually presents with hypercellular bone marrow and anemia. There may be leukopenia and/or and thrombocytopenia, but these features do not represent a diagnostic requirement. In the WHO classification RA shows anemia, no or rare blasts in the peripheral blood, isolated erythoid dysplasia with <5% blasts and <15% ringed sideroblasts in the BM. Refractory Anemia with Ring Sideroblasts is a provisional WHO recognised disease entity among the group of myeloproliferative/myelodysplastic crossover syndromes.[1] Other condition is megaloblastic anaemia which may present with pancytopenia.[2-5] Pancytopenia is combination of anaemia, leucopenia and thrombocytopenia. The aetiology of megaloblastic anaemia is diverse but a common basis is impaired DNA synthesis. The most common causes of megaloblastosis are vitamin B12 and folate deficiencies. Cobalamin metabolism and folate metabolism are intricately related, and abnormalities in these pathways may lead to the attenuated production of DNA. So, there is increased worldwide concern about the consequences of folic acid and vitamin B12 deficiencies on health, which includes megaloblastic anemia.[6] The breast-fed infant of a vitamin B12-deficient mother is also at risk of developing severe developmental abnormalities, growth failure, and anemia. Dietary deficiency of vitamin B12 due to vegetarianism is increasing and also causes hyperhomocysteinemia. Elevated methylmalonic acid

and/or total homocysteine have been found to be sensitive markers of vitamin B12-deficient diets.[7] Vitamin B12 deficiency has a broad spectrum of etiology such as inadequate dietary intake, impaired absorption in the setting of either intrinsic factor deficiency (pernicious anemia)and/or generalized malabsorption syndromes such as Crohn's disease, intestinal infestation by the fish tapeworm, illeal resection and hereditary causes such as severe Methylene tetrahydofolate reductase (MTHFR) deficiency, homocystinuria, and transcobalamin deficiency. Inadequate dietary vitamin B12 intake is one of the common causes of deficiency amongst vegetarians as vitamin B12 is known to occur mostly in animal products. Latest research is focused on intermittent vitamin B12 supplementation as well as detecting bioavailability of B12 in fermented vegetarian foods so that appropriate diet to reduce or eliminate this deficiency can be found. Vitamin B12 deficiency is difficult to pick up clinically as it presents with vague complaints such as decreased mental and/or physical work capacity, reduced attention span, memory loss, irritability and low mood.[8,9] Iron deficiency Anemia is also an important and most common cause of nutritional deficiency worldwide[10] and comprises of about 50%cases of anemia.[11]

In developed countries, the prevalence of Iron deficiency anemia has decreased rapidly during the past few decades, but in developing countries like India it is still an important cause.[12-14] Iron plays a vital role in the maturation, growth, and division of cells.[15-18] Unexplained iron deficiency anemia warrants extensive investigations of the gastrointestinal tract.[19] Bone marrow is hepful in its diagnosis and Prussian blue staining on aspirated bone marrow particles is the gold standard for diagnosing iron deficiency.[20-21]

OBJECTIVE

To find association of Refractory Anemia on bone marrow studies and correlate with dietary habits.

MATERIALS AND METHODS

Patients presenting with Chronic indulent Anemia or Refractory Anemia on initial work up requiring bone marrow aspirate and bone marrow biopsy at Era's Lucknow Medical College from October 2009 to September 2011 are included in this study.

The bone marrow aspiration was performed at the posterior superior iliac spine using Klima needle. As bone marrow clots faster than peripheral blood, films are made from the aspirated material at bedside without delay. Remaining amount is transferred to an Ethylene diamine tetraacetic acid (EDTA) vacutainer. Usually 0.2 - 0.3 mL is sufficient for a morphological diagnosis. It has been found that more than 0.3 mL increases the peripheral blood dilution. After taking the marrow for smears, the trephine or the biopsy specimen was obtained by Jamshidi needle.

Before the biopsy specimen was transferred to 10% neutral buffered formalin, touch imprints were made by gently rolling the bony core across a clean glass slide. This along with the aspiration smears were stained with Leishmann stain. Special stains - Periodic acid Schiff stain, Myeloperoxidase, Sudan black and Perls' stains were used wherever indicated.

Bone marrow aspirate smear

The bone marrow aspirate smear is a preparation designed to spread the cellular material of the marrow so that Leishmann stain can reveal essential cellular details. The bone marrow aspirate is evaluated for: 1), Cellularity of the fragments; 2), Erythropoiesis - cellularity, maturation pattern and any cytological abnormalities; 3), Myelopoiesis - cellularity, maturation pattern and any abnormalities; 4), M:E(Myeloid:Erythroid) ratio; 5), Megakaryopoiesis - number, morphology, presence of immature forms; 6), Lymphocytes; 7), Plasma cells; 8), Parasites/Abnormal cells/Granulomas/Storage cells.

The cellularity was assessed by estimating the percentage of hematopoietic cells compared to fat spaces in the bone marrow. The categories were identified in the following way, depending upon the age of the patient:

- 1), hypercellular: > 75% cells
- 2), normocellular: 25-75% cells
- 3), hypocellular: < 25% cells

Bone marrow biopsy

The microscopic examination of trephine biopsy is best for determining the overall cellularity and presence of infiltrates. Touch imprint preparations from the biopsy material are also useful for morphology. Trephine Biopsy Interpretation - Hematoxylin and eosin sections were studied as follows: adequacy of the biopsy - the ideal specimen should be 1 - 2 cm in length, should not be distorted and should have at least 5 well preserved intertrabecular marrow spaces for interpretation.

RESULTS

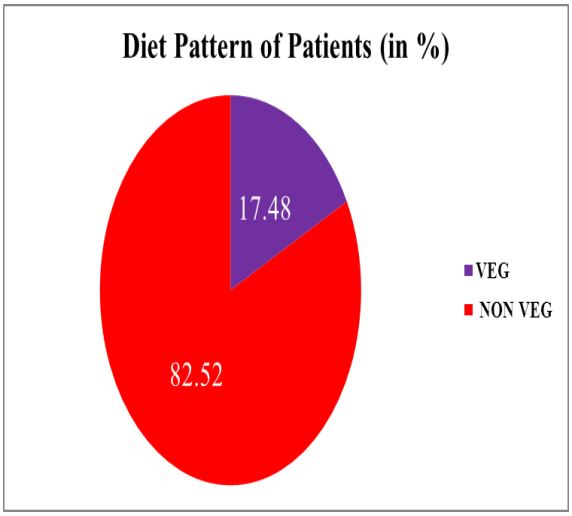

Figure 1: 82.52% patients are non-vegetarian and 17.48% patients are vegetarian.

DIET PATTERN		
DIET	NUMBER OF PATIENTS	%
VEG	50	17.48
NON VEG	236	82.52
Chi Sq = 120.96, p < 0.0001		

Figure 2: Table shows a significant *p* value

A total of 286 presented to us for bone marrow examination. Out of which 236 that constituted 82.52% of total patients, Were non-vegetarian and 50 that constituted 17.48% of total patients, were Vegetarian. It was observed in our study that there are no implications of dietary pattern on prevalence of Refractory Anemia as subjects included in this study were 286; out of which 82.52% were non-vegetarian and 17.48% were Vegetarian, yet studies have shown that Nutritional Anemia is more common in vegetarians. *p* value came out to be < 0.0001, which is significant.

DISCUSSION

Anemia is the most common of all blood disorders. The term "Anemia" is derived from a greek word and it means a deficiency of blood. Historically, the myelodysplastic syndromes (MDS) have been referred to as oligoblastic leukemia, refractory anemia, smoldering acute leukemia, or preleukemia. Refractory anemia is an anemia which does not respond to treatment. Iron deficiency anemia may also be refractory as a clinical manifestation of gastrointestinal problems which disrupt iron metabolism. Patients presented to us with the complaint of Refractory Anemia, which is unresponsive to treatment, were evaluated with the help of Bone Marrow studies. As patients of nutritional anemia also present with very vague Symptoms, sometimes diagnosis becomes difficult. Among all the causes of Anemia diet is a very important risk factor. Other important causes include Thyroid disorders, Lead toxicity, Alcoholism and drugs such as NSAIDs. Nutritional deficiency can be overcome by cooking in iron pots and pans, taking vitamin C rich diet as it helps in absorption of iron and medications for treatment of anemia.

FIGURES

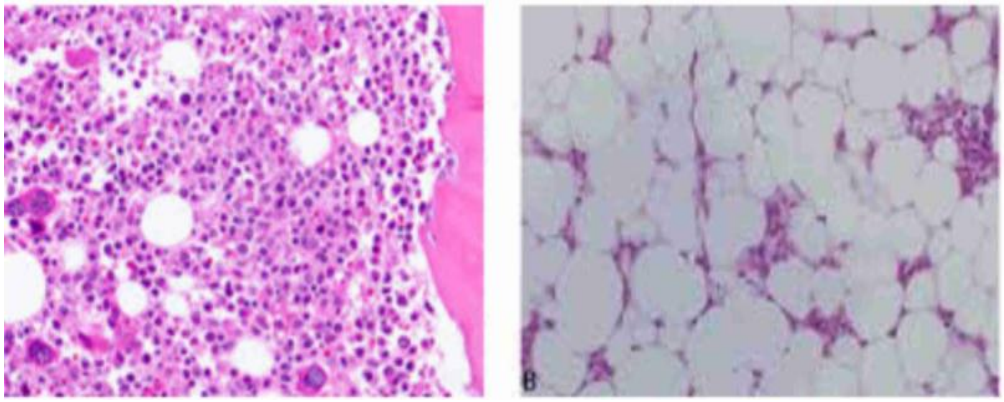

Figure 3: Bone Marrow Biopsy-Healthy Patient, Bone Marrow Biopsy- Aplastic Anemia Patient.

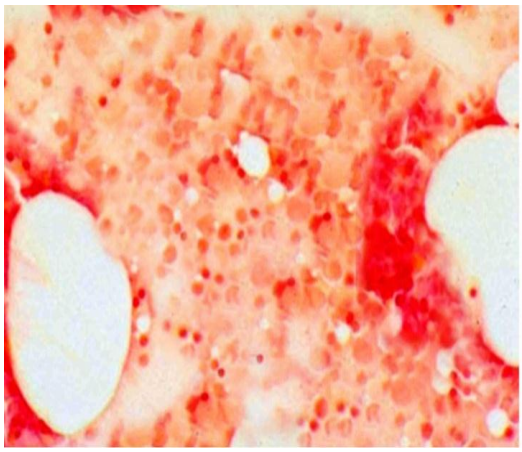

Figure 4: Prussian blue stain for iron in apatient with iron deficiency anemia shows nil iron stores.

CONCLUSION

In our study, it was found that in cases of Refractory Anemia, other etiological factors should be considered primarily than just dietary pattern as it is presumed that vegetarian individuals are more prone to develop Anemias but cases of Refractory Anemias should be categorized separately than Nutritional Anemias and investigated in deep for other etiological factors.

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