INTRODUCTION

Refractory anemias (RA) refer to those anemias unresponsive to most of known lines of therapy except blood transfusion. The term refractory anemia, although in usage a long time, it may be confusing term to those who are not hematologists, because it means more than what it says (*Cuneo and Castoldi, 2003).*

Myelodysplastic syndrome is a heterogeneous group of acquired hematopoietic stem cell disorders characterized by peripheral blood cytopenia, ineffective and dysplastic hematopoiesis, and a varying propensity to leukemic transformation. Refractory anemia is a component of each of myelodysplastic syndrome *(Corey et al., 2007).*

Until recent years, treatment of myelodysplastic syndrome was limited to supportive and palliative care except in the case of the younger patient with a good performance status who could undergo allogeneic stem cell transplantation, and had a suitable donor *(Steensma and Tefferi, 2007).* In refractory anemia patients, stem cell transplantation resulted in a prolonged disease free survival in more than 50% of patients transplanted early in the course of the disease (*Anderson, 2000).*

Refractory aplastic anemia and myelodysplastic syndromes are rare acquired bone marrow failure disorders in childhood. In the last decade, refractory aplastic anemia has shown to be associated with autoimmune phenomena. The use of immunosuppressive therapy has proven to be of great value in both adults and children with severe aplastic anemia *(Fuhrer et al., 2005 and Pongtanakul et al., 2008).*

Refractory autoimmune cytopenias in children include refractory idiopathic thrombocytopenic purpura and refractory autoimmune hemolytic anemia which result from immune mediated destruction of platelets, red cells or both. When traditional therapies as first line agents are ineffective, or clinically stable status can be maintained only by continuing steroids at the risk of long-term side effects.Many approaches exist, and choices for severe and refractory disease include combination strategies. rituximab, splenectomy, vincristine, azathioprine/6-Mercaptopurine, all-trans retinoic acid, mycophenylate mofetil, danazol, and in severe cases, cyclophosphamide. Few comparative studies exist to determine a "best next-line" therapy for these patients *(Zhang et al., 2006 and George, 2006).*

Iron deficiency anemia is the most common type of anemia worldwide, refractory iron deficiency anemia accounts for about 15% of all iron deficiency anemia.

The evidence for causal association between helicobacter pylori and refractory iron deficiency anemia is emerging (*Ashorn et al., 2001).* Iron deficiency anemia may be also refractory as a clinical manifestation of gastrointestinal problems which disrupt iron metabolism *(Mody et al., 2003).*

Micronutrients consist of vitamins and minerals required by the body in small quantities for the normal function of cellular metabolic processes. Micronutrient deficiencies are important nutritional problems and are widespread in many developing countries .When laboratory testing reveals anemia, the clinician needs to investigate whether nutritional imbalance is an underlying cause. Nutrient deficiencies are found in a large variety of anemias and may be the cause of refractory anemia *(Black, 2003).*

Refractory Anemia may be the first recognized manifestation of an endocrine disorder. Anemia resulting from endocrine disease generally is mild to moderate. The pathophysiologic basis of the anemia seen in endocrine disorders is not well understood. However a direct influence of hormones on erythropoiesis may contribute to the development of anemia *(Zimmermann and Kohrle, 2002).*

AIM OF THE WORK

The aim of this study is to give a comprehensive review on refractory anemias in children and to revise and summarize possible causes, classifications, disease evolution and different lines of therapy.

HISTORICAL BACKGROUND

The term refractory anemia was used to describe a group of patients with anemia of unknown etiology and characterized by failure to respond favorably to the exhibition of substances which cure the great majority of cases of anemia *(Bomford and Rhoads, 1941).*

With improved diagnostic techniques and better understanding some of the patients would now be identified as having aplastic anemia, myelosclerosis, sideroplastic anemia, or congenital dyserythropoietic anemia. These cases apart, there remains a syndrome of primary refractory anemia characterized by a qualitative disturbance of erythropoiesis with morphologically and functionally abnormal erythroid cells and also with a variable degree of disturbed myelopoiesis *(Lewis et al., 1977).*

Two types of chronic refractory cytopenia have been recognized in which general hematopoietic defect are particularly prominent. Sometimes known collectively as myelodysplasic syndromes these are refractory anemia with excess of myeloblasts *(Dreyfus et al., 1969 and Duhamel et al., 1976)*, and refractory anemia with proliferative dysplasia *(Gordon-Smith, 1969).*

Refractory anemia with excess of myeloblasts is found in elderly patients who present with symptoms due to pancytopenia. The bone marrow is of normal or

increased cellularity with 30-50% myeloblasts and promylocytes, and one view is that the disorder may be a form of leukemia or smouldring acute leukemia *(Najean et al., 1976 and Ricci et al., 1978)*.

Culture studies in the laboratory can be used to distinguish those cases of refractory anemia with excess of myeloblasts that are essentially benign from those which are preleukaemic; in the benign form the capacity for forming colonies is reduced but the colonies that do form are usually normal, with a normal cluster-to-colony ratio. In the preleukaemic variant there is an alteration in the growth pattern and modification of cluster-to-colony ratio often several months before any clinical or hematological features of leukemia become apparent *(Faille et al., 1978 and Milner et al., 1977)*.

The second type of refractory anemia, proliferative dysplasia, also occurs in elderly patients and presents with pancytopenia and reticulocytopenia. The bone marrow is of normal cellularity with no increase in the proportion of myeloblasts; the unusual features are a marked degree of dyserythropoiesis and an increased amount of reticulum, though there is no obvious fibrosis. The first impression is of a myeloproliferative disorder, but ferrokinetic studies show an aplastic pattern, and in the absence of any extramedullary erythropoiesis the most likely explanation is that the disorder is a variant

of aplastic anemia. There is no evidence that it is preleukemic *(Gordon-Smith, 1969).*

Primary refractory anemia probably results from several different pathogenetic mechanisms. In a study of soft agar colony forming assays of bone marrow of a group of patients with refractory anemia cocultured with normal marrow showed three distinctive growth patterns. In one, colony formation was low but not suppressed by normal cells, suggesting a defect intrinsic to stem cell or progenitor. A second group showed normal colony formation, again with no evidence of suppressor activity, suggesting that in these cases the myelopoietic defect was due to an abnormality in the micro-environment. The third group showed low colony formation in the assay and suppression of colony formation by normal marrow, suggesting that this defect may result from suppressor-cell action *(Valera and Good, 1980).*

Similar mechanisms have been shown in aplastic anemia; possibly aplastic anemia and two forms of refractory anemia are part of spectrum of pancytopenia with a common pathogenesis.The extent of overlap between the conditions and of evolution from one to the other remains to be charted *(Kagan et al., 1979).*

NORMAL BONE MARROW

The bone marrow is a spongy material that fills the bones. It contains early blood cells, called stem cells. The bone marrow occupies the cavities of about 85% of the skeletal system and weighs between 1,600 and 3,700 g in the normal adult *(Frisch and Bartl, 1999).*

Functionally, the bone marrow is the major site of blood cell formation (hematopoiesis) in the body, and also serves as an important element of the reticuloendothelial system. The bone marrow normally produces approximately 2.5 billion red blood cells, 1.0 billion granulocytes, and 2.5 billion platelets daily per kilogram of body weight. However, the marrow has an enormous reserve capacity of 5–10 times normal in times of hematopoietic stress *(Riley et al., 2009).*

Hematopoiesis occurs in the cavities of certain bones that contain hematopoietically active marrow tissue (red marrow). Red marrow is widespread in the bones of children, but is restricted to the skull, sternum, scapulae, vertebrae, ribs, pelvic bone, and the proximal ends of the long bones of the extremities in adults *(Travlos, 2006).*

In these cavities, clusters of stem cells and their progeny lie in the extravascular spaces between vast

networks of vascular channels (sinusoids). Cytoplasmic processes extending from the fibroblastoid (reticular) cells covering the advential surface of the sinusoids form a support lattice for hematopoietic cells. The fibroblastoid cells, vascular endothelial cells, macrophages, extracellular matrix molecules, and adipocytes comprise the bone marrow stroma. This network of cells is important in directing many of the soluble chemical factors (cytokines) that regulate and control the process of hematopoiesis, in addition to providing an adhesive framework onto which the developing cells are bound *(Smith, 2003).*

These factors are referred to collectively as the hematopoietic microenvironment. An analogy is to think of the stem cell as the seed and the microenvironment as the soil. Both must be present for the crop (mature bone marrow cells) to grow successfully *(Gordon, 1994 and Smith, 2003).*

Hematopoietic cells are not arranged at random, but have a consistent spatial relationship to the other components in normal samples. Early myeloid precursors are found adjacent to the endosteal surface, while mature myeloid cells, erythroid cells, and megakaryocytes are found in the central intratrabecular region in association with the marrow sinusoids. However, this arrangement can be easily disrupted by

pathologic states *(Travlos, 2006 and Frisch and Bartl, 1999).*

The cellularity of the bone marrow is defined as the proportion of cellular elements relative to adipose tissue. The cellularity is variable and normally decreases with age. The cellularity for a given age can be approximated by subtracting the patients age from 100. The term hypocellular is used for a bone marrow with decreased cellularity and increased adipose content, while a bone marrow with an increased proportion of hematopoietic cells, and decreased amount of fat, is referred to as hypercellular *(Brown and Gatter, 1993).*

There are other age-related changes in the bone marrow, including a reduction in the volume of trabecular bone and the number of endosteal cells, osteocytes, and paratrabecular sinusoids *(Frisch and Bartl, 1999).*

The cellularity of the bone marrow is not static. Cellularity can rapidly increase in response to stress, infection, or other diseases, while decreased cellularity for age is usually the result of a pathologic disease *(Brown and Gatter, 1993*).

Blood cells (i.e., red cells, white cells, and platelets) are continuously renewed in the bone marrow from progenitors of primitive mesenchymal cells known as

pleuripotential hematopoeitic stem cells (HSCs) (Figure 1). The essential properties of the stem cell are self-renewal and self-differentiation. Progenitor cells arising from the HSC are multipotent, but their progeny are committed to differentiation along the erythroid, granulocytic, monocytic, megakaryocytic, or lymphocytic pathways. Although HSCs form a very small proportion of the total hematopoietic cells, they are maintained throughout the life of the individual *(Frey, 2007).*

Most HSCs are in a reserve, nondividing pool, while a small fraction continually divide but do not differentiate. HSC survival, self-renewal, proliferation, and differentiation require appropriate hormone-like chemical signals (hematopoietic cytokines) and the "microenvironment" provided by the stromal cells and extracellular matrix of the bone marrow *(Weissman, 2000).*

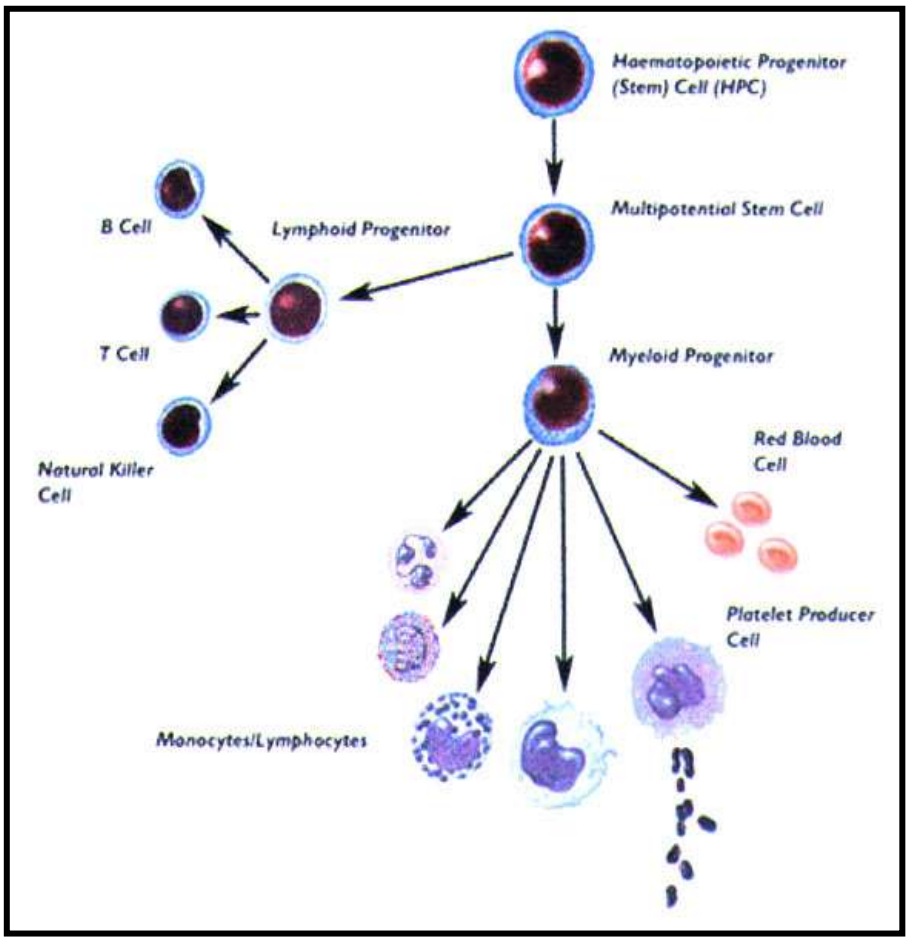

Figure (1): Haematopoietic stem cells *(Meyer et al., 2006)*.

Hematopoietic cytokines are glycoproteins that can be subdivided into two types based on whether they consist of four or six a-helices. Type I hematopoietic cytokines include interleukins (IL) -2, -3, -4, -5, -6, -7, -9, -11, -12, -13, 15, -21, -23, erythropoietin (EPO), thrombopoietin (Tpo), leukemia inhibitory factor, neurotrophin- 1/B-cell-stimulating factor-3, and colony-stimulating factors for granulocytes (G-CSF), and granulocyte–macrophages (GM-CSF). Interferons (IFN)

and IL-10, -19, -20, -22, and -24 are members of the type II cytokine family *(Saharinen et al., 2002).*

Hematopoietic cytokines are secreted by various cells and tissues throughout the body and regulate the production and function of hematopoietic cells *(Saharinen, 2002 and Juul et al., 2007).*

The regulation of hematopoiesis is a complex process that is incompletely understood. The details of this process have been derived primarily from in vitro cell culture experiments that analyze the molecular, immunologic, biochemical, and morphologic effects of various cytokines on different populations of hematopoietic cells. The cytokines exert their biologic effects through interaction with specific receptors on hematopoietic cells that form the hematopoietic receptor superfamily *(Saharinen, 2002 and Nervi et al., 2006).*

Members of the hematopoietic receptor superfamily are membrane glycoproteins composed of structurally similar extracellular domains, single transmembrane domains, and intracellular domains that are constitutively associated with different signaling proteins, the most critical being members of the Jak and Btk/Tec nonreceptor tyrosine kinase families (i.e., Jak-1, Jak 2, Jak-3, and Tyk-2) *(Saharinen, 2002).*

The genes for many of the hematopoietic receptors reside in a small region on the long arm of chromosome 5. Genetic alterations of the hematopoietic receptors or related signaling components result in immunologic and hematologic diseases, including immunodeficiency and leukemia *(Rily et al., 2009).*

Erythroid precursors

Erythropoiesis is a complex, highly regulated process that results in the differentiation of hematopoietic stem cells into mature red blood cells *(Bessis, 1977).*

In the adult, this process normally results in the production of approximately 10^{10} red blood cells per hour to maintain the red blood cell count within a narrow physiologic range (Figure 2). Primitive erythroid precursors arise from the pluripotent stem cell and subsequently undergo a progressive series of structural and biochemical changes driven by EPO and other hematopoietic cytokines. Erythroid colony forming units (CFU-E) arise under the influence of EPO. The pronormoblast results from cell division of the CFU-E, and is the first morphologically identifiable red blood cell. Subsequent cellular stages in erythropoiesis include the basophilic normoblast, polychromatophilic normoblast, orthochromatic normoblast, reticulocyte, and mature red blood cell *(Riley et al., 2009).*

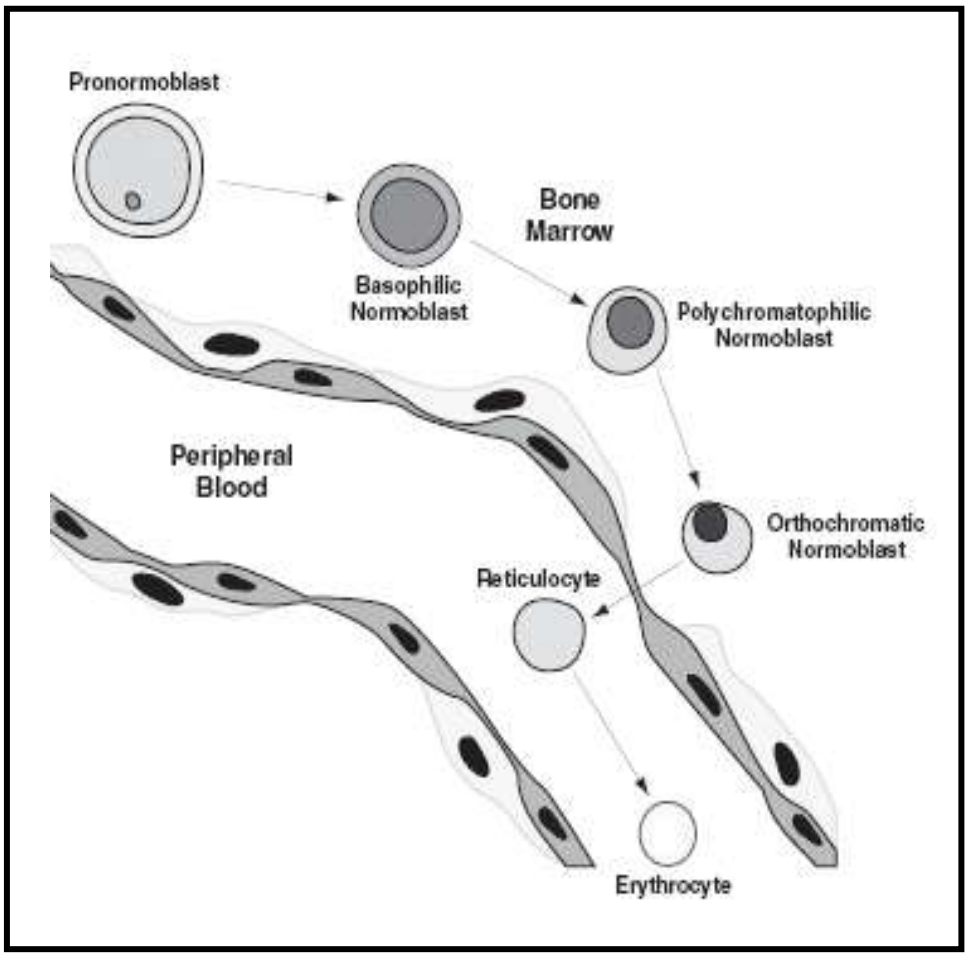

Figure (2): Eryththropoiesis. Illustration of Stages of erythroid development *(Riley et al., 2009).*

Together, the red blood cells and the various morphologically identifiable red blood cell precursors comprise the erythron. The progression from a pro-normoblast to a nonnucleated red blood cell requires 3–5 days but can be accelerated if biologically needed. The erythroid precursors are described below (Figure 3). The term "normoblast" should only be used in reference to normal erythropoiesis. The more general