SERUM 25-HYDROXYVITAMIN D LEVELS: RELATION TO DISEASE STATUS AND PROGNOSIS IN ACUTE MYELOID LEUKEMIA

Thesis

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By

Mai El-sayed Kamal Mohmed

MB, BCH

Ain Shams University

Supervised by

Professor/ Tahani Ali El-kerdany

Professor of Clinical and Chemical Pathology

Faculty of Medicine-Ain Shams University

Doctor / Doaa Ahmed Gamal Eissa

Lecturer of Clinical and Chemical Pathology

Faculty of Medicine-Ain Shams University

Faculty of Medicine - Ain Shams University

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كلية الطب –جامعة عين شمس

الدكتور / دعاء احمد جمال عيسى
مدرس الباثولوجيا الاكلينيكية والكيميائية
كلية الطب –جامعة عين شمس

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INTRODUCTION

Vitamin D, a steroid hormone produced in skin, acts through a nuclear transcription factor to regulate many aspects of cellular growth and differentiation, and exerts its action via specific intracellular receptors, which are found in normal as well as cancer cells ***(Holick, 2007)***.

Serum levels of 25-hydroxyvitamin D (25[OH]D) reflect whole-body vitamin D stores and are used to assess individual vitamin D adequacy or insufficiency. 25(OH)D is converted to 1,25-dihydroxyvitamin D (1,25[OH]2D), the physiologically active form of vitamin D, via the action of 1-α-hydroxylase primarily in the kidney. Once formed, 1,25(OH)2D exerts its biologic effects by binding to the vitamin D nuclear transcription factor receptor, which regulates the expression of nearly 200 genes ***(Carlberg, 2003)*.**

Several reports now suggest that low serum 25(OH)D levels may be associated with increased incidence of colorectal, breast and other cancers ***(Yin et al., 2009 and Crew et al., 2009).*** In addition to the risk of developing malignancy, recent data suggest that low 25(OH)D levels at diagnosis may be associated with poorer prognosis in colorectal, breast, melanoma, and lung cancer, although these data have not yet been replicated in independent

cohorts ***(Ng et al., 2008; Goodwin et al., 2009 and Newton-Bishop et al., 2009).***

Lower levels of circulating 25(0H)D appeared to be related to a progressive stage in acute leukemias and poor response to therapy and therefore, to the aggressiveness of the disease. It is a prognostic marker in patients with acute leukemias ***(Thomas et al., 2011).***

AIM OF THE WORK

This study aimed to measure 25-hydroxyvitamin D levels in acute myeloid leukemia patients and correlate these levels with disease status and standard prognostic markers of the disease.

Chapter (1):
ACUTE MYELOID LEUKEMIA

Definition:

Acute myeloid leukemia (AML) is a hematopoietic stem cell disorder characterized by a block in differentiation of hematopoiesis, resulting in growth of a clonal neoplastic cells or blasts **(*Shipley and Butera, 2009*).**

The cell of origin in AML is a blast that most often show commited differentiation. In approximately 5 to 10% of patients blasts have erythroid or megakaryocytic differentiation. For this reason, acute non-lymphoblastic leukemia (ANLL) has been considered a more precise term but AML is more common and is the recommended term ***(Cheson et al.,2003).***

Epidemiology:

Acute myeloid leukemia (AML) has an incidence of 2-3 per 100,000 in children rising to 15 per 100,000 in older adults. It can occur at all ages but has its peak incidence in seventh decade ***(Burnett, 2005).***

AML accounts for 15 to 20 percent of the leukemias in children and 80 percent of acute leukemias in adults. It's slightly more common in males. Little difference in incidence is seen between individuals of African or

European descent at any age, while a lower incidence is seen in persons of Asians descent. An increase in frequency of AML is seen in Jews, especially those of Eastern European descent ***(Liesveld and Lichtmam, 2006).***

Etiology and risk factors

Although the cause of AML in adults is unknown, a variety of hereditary and environmental factors appear to play an etiologic role ***(Larson, 2007).***

I- Hereditary factors

An increased incidence of AML is seen in patients with disorders associated with chromatin fragility such as Bloom syndrome, Fanconi anemia, and Kostmann syndrome, and with Wiskott-Aldrich syndrome or ataxia telangiectasia syndrome. Other syndromes, such as Down (trisomy of chromosome 21), Klinefelter (XXY and variants), and Patau (trisomy of chromosome 13), have also been associated with a higher incidence of AML ***(Jabbour et al., 2006).***

II- Environmental factors

a- Smoking:

The only proven lifestyle-related risk factor for AML is smoking. Scientists estimate that as many as 1 out of 5 cases of AML are caused by smoking

(http://www.nlm.nih.gov/medlineplus/ency/article/000542.htm).

b- Chemical exposure:

The risk of AML may be increased by exposure to certain chemicals **(http://www.nlm.nih.gov/medlineplus/ency/article/000542.htm)**.

Benzene is an established Human leukemogen ***(Zhang et al., 2005)***. It causes leukemia, aplastic anemia, and other bone marrow disorders in humans ***(Zhang et al., 1996).***

The precise mechanism by which benzene induces these effects is unclear, but its conversion to metabolite is essential. Benzene is metabolized in the liver by cytochrome P450 2E1 (CYP2E1) to benzene oxide, which rearranges non-enzymatically into phenol. Phenol can either be conjugated to a sulfate or glucoronide or be hydroxylated to catechol, hydroquinone and 1, 2, 4-benzetriol. It is believed that these polyphenolic metabolites then travel to the bone marrow, where they are oxidized to highly toxic quinines by myeloperoxidase and produce hematotoxic and leukemogenic effect ***(Smith et al., 2000).***

c- Radiation exposure:

Therapeutic radiation increases AML risk, particularly if given with alkylating agents ***(Jabbour et al., 2006).***

AML is also common in workers in the nuclear industry but not in people living near nuclear power plants. Current use of diagnostic x-ray imaging does not appear to be associated with any increased leukemia risk in patients. However, fetal exposure to intrauterine x-rays increases the risk of subsequent childhood leukemia ***(Larson et al., 2007).***

The primary carcinogenic effect of ionizing radiation is causing radiation induced genomic instability in hemopoeitic cells. However, the relationship of inducible instability and AML induction is still unexplained ***(Larson, 2007).***

Exposure to electromagnetic fields (such as living near power lines), some dyes, herbicides and pesticides, has been implicated as another potential risk factor ***(Konoplev and Bueso-Ramos, 2006).***

d- Drugs

It is now recognized that there are two types of chemotherapy-related leukemias: (a) the classic alkylating

agent-induced type (eg. Cylophosphamide, melphalan and nitrogen mustard) in which the leukemia is preceded by a myelodysplastic prodrome and (b) an epipodophyllotoxin-associated type (agents that inhibit the DNA repair enzyme topoisomerase II eg. etoposide and teniposide) with shorter incubation period that is associated with myelomonocytic or monocytic differentiation ***(Konoplev and Bueso-Ramos, 2006).***

Drugs such as chloramphenicol, phenylbutazone, chloroquine, and methoxypsoralen can induce marrow damage that may later evolve into AML ***(Jabbour et al., 2006).***

An excess risk of AML has been previously reported in health occupations, for example, physicians or nurses handling anti-neoplastics drugs and pathologist being exposed to formaldehyde ***(Salamanchuk et al., 2004).***

III- Acquired diseases

Most secondary cases of AML occur within 10 years after treatment of Hodgkin disease, non-Hodgkin lymphoma, or childhood acute lymphoblastic leukemia (ALL). Secondary leukemias also sometimes occur following treatment of breast, ovarian, or other cancers ***(http://www.nlm.nih.gov/medlineplus/ency/article/000542.htm).***

AML may be also secondary to the progression of a myelodysplastic process or due to progression of a chronic bone marrow "stem cells" disorder or chronic myeloproliferative disorders (MPD), such as polycythemia vera, essential thrombocythemia, paroxysmal nocturnal hemoglobinuria, or idiopathic myelofibrosis ***(Konoplev and Bueso-Ramos, 2006).***

There are many biologic and clinical similarities between myelodysplastic syndrome (MDS) and the more common subtypes of AML that are seen in older adults, and some patients may have disease that evolved from unrecognized MDS ***(Konoplev and Bueso-Ramos, 2006).***

Pathogenesis:

Acute myeloid leukemia is believed to begin in a single somatic hematopoietic progenitor that transforms to a cell incapable of normal differentiation. Many of these cells no longer posess the normal property of apoptosis resulting in a cell with prolonged life-span and unrestricted clonal proliferation. A Major cause of morbidity is the deficiency of normal functioning mature hemopoietic cells, rather than the presence of numerous malignant cells ***(Weinblatt, 2006).***

Development of AML results in a block differentiation, increased proliferation and inhibition of

apoptosis. This has been hypothesized to be due to multiple genetic events, activation or inappropriate expression of surface membrane hemopoietic growth factors ***(Zhou et al., 2000).***

1. Genetic Factors:

Somatic mutation results from chromosomal translocation in nearly 80 percent of patients results in rearrangement of a critical region of proto-oncogene. The fusion of portions of two genes usually doesn't prevent the process of transcription and thus the fusion gene encodes a fusion protein that, because of its abnormal structure, disrupts a normal cell pathway and leads to malignant transformation of the cell. This protein is often a transcription factor that disrupts the regulatory sequences that control differentiation, growth rate or survival of blood cell progenitors ***(Kelly and Gillilanti, 2002).***

These primary mutations are not sufficient to cause AML. Addition activating mutations, for example, in hematopoietic tyrosine FLT3 and KIT or N-Ras and K-Ras are required to induce a proliferative advantage in the affected primitive cell. Other mutations occur in leukemic cells involving, myloproliferative leukemia virus oncogene (MPL), Retinoblastoma (Rb), Wilm's tumor gene (WT1) and P53. The most common occurring cytogenetic abnormalities in AML include: t(15;17), t(8;21), inv(16),

+8, +21, del(5q), -7, -8, 11q23 translocation and 12p11-13 abnormalities. Interaction with loss of function mutations in hematopoietic transcription factors probably causes the acute leukemia phenotype characterized by disordered proliferation, programmed death, differentiation and maturation stop ***(Liesveld and Lichtman, 2006).***

2. Hematopoietic growth factors and cytokines:

Leukemic cells exhibit a proliferative response to many of the endogenous hematopoietic growth factors critical for normal hematopoiesis such as G-CSF, GM-CSF, M-CSF, CSF, IL-3, IL-4, IL-5, IL-6, **IL**-7, FLT3 and KIT ligand which are mediated through specific growth factor receptors that are frequently expressed on the surface of ANLL cells ***(Rosenfeld and List, 2001).***

It appears that a combination of these factors can produce a synergistic growth response. In particular, stem cell factor can enhance by some of 10-20 folds the proliferation of leukemic blasts induced by G-CSF, GM-CSF and IL-3 ***(Giles et al., 2002).***

Activation of surface membrane receptors leading to the proliferation of ANLL cells may occur in multiple ways:

a. Over expression of growth factors receptors and their interaction with normal endogenous hematopoietic growth factors:

The interaction with specific Human growth factors (HGFs) with their corresponding reeceptors presumably triggers a cascade of molecular events leads to stimulation of cells division ***(Rosenfeld and List, 2001)***

Hepatocyte growth factor is a pleiotropic cytokine involved in hepatocyte morphogenesis is secreted by stromal cells. In conjunction with other growth factors (GM-CSF, IL-3), it can augment the growth of committed progenitors through interaction with its receptors found on CD34+ cells ***(Weimer et al., 1998).***

b. Interaction with autocrine growth factors:

Autonomous growth has been reported to occur as a result of autocrine or paracrine stimulation of a number of these HGFs. The acquisition of autonomous growth capability AML cells to be more aggressive by making them independent of stromal cell production of essential growth factors ***(Dang et al., 1997).***

There is evidence that secretion of IL-1 can stimulate the release of G-CSF, GM-CSF from endothelial cells which in turn may affect the proliferation of leukemic blasts (autocrine growth factor) ***(Giles et al., 2002).***

c. Activation of mutation within the receptors themselves:

Disruption of normal hematopoietic growth factors signal transduction pathways and mutations in hematopoietic growth factors receptor genes that interfere with normal receptor complex formation may lead to their activation in absence of specific ligands. Mutation in the G-CSF, FLT3 and c-KIT receptor genes have been described in AML ***(Lowenberg and Burnett, 2005).***

3. Adhesion molecules:

Evidence indicates that in addition to cytokines, adhesion molecules are involved in mediating signal transduction in hematopoietic precursors and hence play an important role in cell proliferation that extend beyond mediating hemopoietc cell contact and adhesion to stromal cells and extracellular matrix (ECM) ***(Levesque and Simmons, 1999).***

Primitive HSCs express a wide range of cell adhesion molecules, which include members of the integrin, selectin and immunoglobulin families ***(Wong et al., 2007).***

4. Molecular factors:

a. Proto-oncogenes

Proto-oncogenes are genes responsible for growth of target cells. So if they are activated with increasing capacity, this may lead to uncontrolled proliferation, block of normal differentiation , prevention of apoptosis, and thus