CCL3 (MIP-1A) PLASMA LEVELS AND THE RISK FOR DISEASE PROGRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA

Thesis

Submitted for Partial Fulfillment of Master Degree
of **Clinical and Chemical Pathology**

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2012

مستوى السي سي ال ٣ في الدم و علاقته بمخاطر تطور مرض ابيضاض الدم الليمفاوي المزمن

رسالة

توطئة للحصول على درجة الماجستيرفي

الباثولوجيا الاكلينيكية و الكيميائية

مقدمة من

الطبيب/ فادي أشرف فرنسيس

بكالوريوس الطب والجراحة

كلية الطب- جامعة عين شمس

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كلية الطب

جامعة عين شمس

2012

Acknowledgment

First and foremost, I thank **GOD** *for helping and guiding me in accomplishing this work.*

I would like to express my sincere gratitude to ***Professor Dr. Tahany Ali El Kerdany,*** *Professor of Clinical pathology, Ain Shams University, for her great support and stimulating views. Her active, persistent guidance and overwhelming kindness have been of great help throughout this work.*

I must extend my warmest gratitude to ***Dr.Soha Raouf Youssef,*** *Professor of Clinical pathology, Ain Shams University, for her great help and faithful advice. Her continuous encouragement was of great value and support to me.*

Last but definitely not least, I would like to thank my parents for their guidance and for all the suffering and hardships I made them face from the day I entered this world. To them I owe my life. And I would like to thank my wife

Suzanne Nabil for being always there for me, giving me hope strength and patience. Tomorrow with you is better.

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INTRODUCTION

Chronic lymphocytic leukemia (CLL) is a monoclonal disorder characterized by a progressive accumulation of functionally incompetent lymphocytes. It is the most common form of leukemia found in adults in Western countries ***(Elter et al., 2006).*** The cells of origin in most patients with CLL are clonal B cells arrested in the B-cell differentiation pathway, intermediate between pre-B cells and mature B cells. Morphologically in the peripheral blood, these cells resemble mature lymphocytes ***(Zeng et al., 2009).***

In CLL, the DNA of a B cell is damaged, so that it cannot produce antibodies. Additionally, B cells grow out of control and accumulate in the bone marrow and blood, where they crowd out healthy blood cells. CLL is a stage of small lymphocytic lymphoma (SLL), a type of B-cell lymphoma, which presents primarily in the lymph nodes. CLL and SLL are considered the same underlying disease, just with different appearances ***(Harris et al., 1997).***

Although not necessary for the diagnosis or staging of CLL, molecular testing now exists that may help predict prognosis or clinical course. A new marker, CCL 3, is a chemokine secreted by CLL cells in response to B cell receptor activation. This marker fosters interactions between CLL cells and the leukemia microenvironment.

CCL3 secretion correlates with expression of the 70-kDa ζ-associated protein (ZAP-70) and responsiveness of the CLL clone to (BCR) stimulation, hence its level in plasma correlates with disease progression and can predict the prognosis of the condition ***(Sivina et al., 2011).***

AIM OF THE WORK

This study aims to measure the serum level of CCL 3 in CLL patients and study its utility in predicting progressive disease.

CHRONIC LYMPHOCYTIC LEUKEMIA

I- Definition:

Chronic lymphocytic leukemia is a clonal lymphoproliferative disorder characterized by progressive accumu-lation of morphologically and immunophenotypically mature lymphocytes. This disease is related to the accumulation of monoclonal B cells with the morphology of small mature lymphocytes. Typically, B-CLL cells exhibit a characteristic immunophenotype, co-expressing CD19, CD5 and CD23, in the absence or low expression of surface CD22, CD79b and FMC7 ***(Gachard et al., 2008).***

II- Epidemiology:

A) Prevalence and Incidence:

The B-CLL accounts for 25% to 30% of all adult leukemias. In Asian countries, CLL represents only 5% of leukemias, with the T-cell phenotype predominating. This geographic difference in incidence is most likely the result of genetic factors ***(O'Brien and Keating, 2005).***

B) Age and sex:

The prototype is a male with a median age of 65 years and its development increases progressively with age. It is 2.8 times higher for older men than older women. Less

than 10% of patients are seen in persons 40 years old or younger and infrequent in children ***(Catovsky et al., 2004).***

C) Predisposing factors

1- Environmental:

Environmental factors do not appear to play a role in the pathogenesis of B-CLL. The incidence of CLL was not associated with exposure to pesticides, sunlight, ionizing radiation or known carcinogens ***(Wierda et al., 2008)***.

The risk of CLL is not increased in those with immunodeficiency syndromes and in persons exposed to electromagnetic waves ***(Jacobs and Wood, 2002).***

2- Occupational Factors:

A higher incidence of CLL is seen in some groups of workers in the rubber industry. The chemicals used in this industry that are linked to the development of CLL include carbon tetra-chloride, carbon disulfide, acetone and ethylacetate. The duration and level of exposure to these chemicals appear to correlate with the risk of developing leukaemia ***(Rai and Gupta, 2003).*** Specific agricultural exposures linked with elevated risk of CLL include DDT, animal breeding and working in flour mills ***(Zheng et al., 2002).***

3- Infections:

Antibodies specific for type C hepatitis virus (HCV) and/or viral DNA have been identified in some patients, suggesting a pathogenic role. However, some studies have failed to verify an association between the development of CLL and infection with HCV. CLL cells are resistant to infection with Epstein Barr virus (EBV), except in unusual cases, making it unlikely that EBV plays a pathogenic role ***(Hsieh et al., 2002).***

4- Hereditary and Genetic Factors:

First degree relatives of patients with CLL are more than three times at risk for having this disorder or other lymphoid neoplasms than is the general population and often present at a younger age ***(Sgambati et al., 2001).*** The genetic factors that contribute to the increased incidence of CLL in certain families are unknown. There is no apparent association between human leukocyte antigen (HLA) haplotype and disease susceptibility ***(Kipps, 2006).***

One interesting phenomenon observed in CLL is anticipation which refers to the worsening severity of earlier age of onset in successive generations ***(Sgambati et al., 2001).***

III- Pathophysiology:

The cells of origin in the majority of patients with CLL are clonal B cells arrested in the B-cell differentiation pathway, intermediate between pre-B cells and mature B cells. B-CLL lymphocytes typically show B-cell surface antigens, as demonstrated by CD19, CD20, CD21, and CD24 monoclonal antibodies, in addition, they express CD5, which is more typically found on T cells. Because normal CD5+ B cells are present in the mantle zone (MZ) of lymphoid follicles, B-cell CLL is most likely a malignancy of a MZ-based subpopulation of anergic self-reactive cells devoted to the production of polyreactive natural autoantibodies ***(Perry, 2003).***

IV- Clinical features:

A- General symptoms:

About 70% of CLL patients are diagnosed in an asymptomatic phase on the occasion of a routine medical examination, while others may present with more severe symptoms as weight loss, recurrent infections, bleeding and/or symptomatic anemia. However, night sweats and fevers (the so called B symptoms) are uncommon and evaluation for complicating infectious diseases should be done ***(Kipps, 2006).***

B- Lymphadenopathy:

Nearly 80% of all patients have non-tender lymphadenopathy at diagnosis. The lymph nodes are usually discrete and freely mobile ***(Kipps, 2001)***. Enlargement of cervical and supraclavicular nodes occur more commonly than axillary or inguinal lymphadenopathy ***(Johnston, 2004)***.

C- Splenomegaly and Hepatomegaly:

Approximately 50% of CLL patients present with mild to moderate splenomegaly. Splenomegaly may result in hypersplenism contributing to anemia and thrombocytopenia. However, in CLL such cytopenias are more commonly secondary to extensive marrow involvement with CLL and/or intermittent expression of auto-antibodies. Hepatomegaly occurs less frequently than splenomegaly ***(O'Brien and Keating, 2005).***

D- Extranodal Involvement:

Organ infiltration with leukemic cells is frequently detected at autopsy but is not commonly symptomatic. However, it may become symptomatic when it develops in certain locations such as in the retro-orbit, pericardium or lung parenchyma ***(O'Brien and Keating, 2005).***

The gastrointestinal tract may be infiltrated by leukemic cells causing mucosal thickening, ulceration and bleeding may occur. Small bowel affection often causes

diarrhea and intestinal malabsorption which induces megaloblastic anemia due to folate malabsorption ***(Monteserrat et al., 1997).***

Leptomeningeal leukemia is rare and if present, is usually seen in patients with refractory disease ***(O'Brien and Keating, 2005).***

E- Immunological Complications:

Patients with CLL have an increased risk for Herpes Zoster infection, as most of them have an acquired immune deficiency. They have greater susceptibility to infection due to numerous factors including hypogammaglobulinemia, low complement levels, functional defects in T cells, altered leukemic cell expression of major histocompatibility complex class II antigens and impaired granulocytic function ***(Francis et al., 2006).***

Patients develop autoimmune hemolytic anemia and immune thrombocytopenic purpura and less frequently, patients may develop pure red cell aplasia or neutropenia secondary to the development of auto-antibodies against marrow hematopoietic progenitor cells. These auto-antibodies are polyclonal and are usually immunoglobulins ***(O'Brien and Keating, 2005).***

V- Laboratory Findings:

A- Peripheral Blood Findings:

1- Lymphocytosis:

The diagnosis of CLL requires a sustained monoclonal lymphocytosis greater than 5000/ul ($5x10^9$/L). At diagnosis, the absolute lymphocyte count generally exceeds 10,000/ul and is sometimes greater than 100,000/ul. The majority of lymphocytes are small with scanty, bluish cytoplasm, clumped nuclear chromatin, inconspicuous nucleolus and absence of azurophilic granules in the cytoplasm ***(Amato et al., 2007).***

Ruptured lymphocytes or "smudge" cells are commonly seen in the peripheral smear, reflecting fragility and distortion during preparation of the peripheral smear on the glass slide ***(Montserrat, 1999).***

The French American British (FAB) classification system divides patients into three groups depending on the percentage of abnormal cells (Table 1). In typical CLL more than 90% of the cells are small (Figure 1); in CLL/PLL, 11% to 54% of the cells are prolymphocytes (Figure 2) and in atypical CLL, there is heterogeneous morphology but 10% of the cells are prolymphocytes ***(O'Brien and Keating, 2005).***