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The Immunological Effect of a Gleevec Drug as Tyrosin Kinase Inhibitor in Auto-immune Thyroid Peroxidase in Iraqi Patients with Chronic Myeloid Leukemia

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Abstract

A gleevec drug a tyrosine kinase inhibitor that targets the so-called Philadelphia chromosome that characteristic of chronic myeloid leukemia (CML) which has been widely used in treatment of several hematological malignancies, and there is concern about the long-term immune effects of its use. Autoimmune disorders in patients treated with gleevec drug may be related to the direct immune modulating properties or may be linked to a possible toxic effect in target organs, triggering autoimmunity diseases. The study described the development of symptomatic autoimmune thyroid peroxidase antibodies (TPO) in 51 patients with CML compared with 10 subjects as healthy control. The samples collected from Iraqi National Center for Research and Treatment of Hematological Diseases/University of Al-Mustansiryiah.

Epidemiological data such as age, sex and cure period were recorded for all of the patients. The range age of the patients were (16-70) years male and female with a cure period (8 months to 10 years) in chronic treatment with recombinant a gleevec drug. The samples tested for anti-TPO by using Enzyme Linked Immune Sorbent Assay (ELISA) which revealed significant differences ($p<0.05$) between control and patients. The patient's samples diagnosed for chronic myelogenous leukemia according to clinical physical examinations, Real-time polymerase chain reaction (RT-PCR) for detection quantification of mRNA chimerical gene *bcr/abl* (*M-bcr*) and mRNA *gene/abl* and by Fluorescence in Situ Hybridization (FISH) techniques in the clinical material. The aim of the study was to test the possible association of the generation of autoimmune disease on thyroid peroxidase by the effect of gleevec drug in patients with CML

Keywords: chronic myeloid leukemia, gleevec drug, autoimmune thyroid diseases, anti -TPO.

التأثير المناعي لعقار الكليفيك المثبط للتايروسين كاينيز في انزيم الثايروويد بيروكسيديز المناعي الذاتي في المرضى العراقيين المصابين بابيضاض النخاع المزمن

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الخلاصة

لقد وصفت الدراسة تطور اعراض اضداد الثايروويد بيروكسيديز Thyroid peroxidase antibodies (TPO) في (51) مريض بسرطان ابيضاض الدم (CML) مقارنة ب (10) اصحاء كسيطرة. المدى العمري للمرضى يتراوح بين (16–70) سنة بين رجل وامرأة بفترة علاج تراوحت (8 اشهر الى 10 سنوات) بعقار

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gleevec . تم اختبار العينات لأضداد Thyroid peroxidase antibodies (TPO) من خلال تقنية الايلايزا Enzyme Linked Immune Assay (ELISA) والتي اوضحت وجود فروق معنوية ($p<0.05$) بين السيطرة الاصحاء والمرضى ، حيث تم تشخيص عينات المرضى لمرض ابيضاض الدم من خلال الفحوصات السريرية البدنية ومن خلال تقنيات سلسلة تفاعل انزيم البلمرة Real Time polymerase chain reaction (RT–PCR) لجين ***bcr/abl*** في الحامض النووي الرايبي الرسول m– RNA وكذلك من خلال تقنية التهجين الموضعي المتفلور Fluorescence in Situ Hybridization (FISH) ضمن العمل السريري. ان الهدف من الدراسة هو اختبار العلاقة المحتملة لتكوين امراض مناعة ذاتية autoimmune disease ضد انزيم التايروسين بيروكسديز autoantibodies (TPO) من خلال تاثير عقار gleevec في المرضى المصابين بابيضاض الدم المزمن (CML) .

Introduction

Chronic myeloid leukemia is a clonal myelo-proliferative disorder of a pluripotent stem cell [1,2]. It was the first malignancy that had a specific chromosomal abnormality uniquely linked to it after the discovery of a minute chromosome now known as the Philadelphia (Ph) chromosome [3] later defined to result at (9;22) reciprocal chromosomal translocation [4]. Critical importance was the demonstration that this translocation involved the *Abl1* (Abelson) proto oncogene in chromosome 9 and the *BCR* (breakpoint cluster region) gene in chromosome 22 [5,6**]**. Patients with CML which create an abnormal gene called *bcr*/*abl* leads to the production of a type of enzyme called a tyrosine kinase, which signals the marrow to make too many white blood cells. Gleevec works by blocking the tyrosine kinase enzyme so that the marrow stops (slows down) making too many white blood cells. For this reason, gleevec and similar drugs are called tyrosine kinase inhibitors (TKIs) [7,8]. With gleevec, a remarkable cancer drug, the approach was to target the disease at the cellular and subcellular level. Gleevec, also marketed internationally as gleevec and sometimes referred to by its chemical name imatinib, entered the medical world with a bang. Imatinib is a small-molecule protein tyrosine kinase inhibitor developed to target the gene product of the Philadelphia chromosome *bcr*/*abl* translocation in chronic myelogenous leukemia (CML). And was initially approved for the treatment of *bcr*/*abl*-positive CML and more recently approved to treat C-Kit–expressing gastrointestinal stromal tumors (GISTs) based on its ability to antagonize C-Kit [7- 9]. Tyrosine kinases (TKs) are important for the regulation of growth, differentiation, survival, and motility of various tumors over express TKs or harbor activating TK mutations leading to uncontrolled mitogenic signals to the neoplastic cells [10-15]. Gleevec (Imatinib) mesylate as an example of this novel class of drugs suppresses the TK activities of c-abl, *BCR-ABL*, platelet-derived growth factor receptor (PDGFR), and c-kit receptors. Also has been approved for use in patients with several types of gastrointestinal tumors, can provide benefit in autoimmune arthritis but has not been previously determined [16-21]. And have many properties in many immunity cases: It inhibits macrophage signal transduction events**,** M-CSF is present in RA synovial tissue and has been shown to exacerbate CIA, inhibits TNF-α production by human RA SFMCs, inhibits mast cell production of proinflammatory cytokines, B cell proliferation and immunoglobulin production in vitro, anti-collagen T cell proliferation and cytokine production [22-26].

Methodology

Fifty one patients male to female ratio (49.02% to 50.98%) range aged (16-70) years were carried out and diagnosed by the consultant medical staff at the National Centre of Haematology of Al-Mustansiryiah University. The diagnosis for CML patients based on; clinical examination, laboratory investigations of complete blood picture, histopathological examination of bone marrow aspirate and biopsy. So as molecular study was done by (RT-PCR) technique (Sacace kit Biotechnologies) for detection quantification of mRNA chimerical gene ***bcr/abl*** (**M-*bcr***) and mRNA gene/ ***abl*** in the clinical material by using Gene Expert diagnosis system as mentioned by this kit, and also molecular genetics by using FISH technique as a confirmative for detection defect gene. **The association of diagnosis tests represented by molecular and FISH tests were confirmed by special privet lab agreement by Ministry of Iraqi Health**. While the immunological test through ELISA technique was done in Educational laboratories center/Medical city. The samples of patients compared with ten healthy subjects as a control group whom which had no signs or symptoms of any type of leukemia as detected by their diagnosis tests and the consequent view point of the consultant medical staff.

Collection of samples:

The samples of CML patients so as a healthy control group of subjects were collected according to age, gender, disease duration and disease phase. The blood of 5ml of samples were collected by venipuncture, they were drawn into three types of tubes: blood of 1.5 ml in EDTA tubes for molecular test, 1.5ml in hepariniezed tubes for FISH technique and 2ml in plane tubes for ELISA tests. The samples related with ELISA test were subjected to centrifugation at 2000 rpm for 10 minutes to collect the sera for assessment of anti-TPO (Aeskulisa company kit). The sera were frozen at -20ºC until the assessment.

Laboratory Investigations:

Anti-TPO membrane-bound glycoprotein of thyroid gland are important for ruling out autoimmune thyroid diseases. Anti-TPO was detected by ELISA kit; this assay system utilizes anti-human immunoglobulin conjugated to horse radish perioxidase. The normal range of anti-TPO as recommended by Aeskulisa is ≤ 40 IU/ ml as mentioned by company kit.

Result and Discussion

Thyroid dysfunction, is a well recognized side effect of treatment with tyrosine kinase inhibitors (TKIs) (27). Gleevec can cause unusual adverse effects, multitargeted of TKIs that have been demonstrated to induce hypothyroidism and thyroid dysfunction. In our study of 51 patients are currently in chronic treatment with a gleevec for hematological malignancies of CML which compared with healthy control there were an effect and there were significant differences ($p<0.05$) as mentioned in Table -1.

Table 1- Comparison between patients and control group in concentration of TPO antibodies (lower and upper) of 40 Iu/ml

Group	No.	Mean (IU/ml)	SE	Min.	Max.	P-value	T-test
Patients	**51**	**27.85**	**15.57**	**0.010**	**775.70**	**0.0435**	**24.72 ***
Control	**10**	**0.0439**	**0.006**	**0.012**	**0.080**		
*** (P<0.05).**							

The median age of the patients were 49.02, percent of male to female which were (50.98 % to 49.01%). And the TPO antibodies appeared to be increasing in progressing with the age, and specially the age of 40 and more, as mentioned in Table -2 which represent significant differences ($P<0.05$), and appeared that the treatment of the patients affected by the age.

Table 2- The Effect of TPO antibodies in age group

Age (year)	No.	Mean (IU/ml)	SE	Min.	Max.	P-value	LSD-value
Less than 30	**4**	**8.55**	**5.01**	**0.012**	**19.26**	**0.053**	**22.84 ***
30-40	**16**	**18.78**	**12.60**	**0.010**	**203.30**		
More than 40	**31**	**35.02**	**138.57**	**0.010**	**775.70**		
*** (P<0.05).**							

Retrospective studies indicate that TKIs can induce hypothyroidism [28, 29]. So as the effectiveness of TKIs on thyroid diseases through autoimmunity, in which the body's immune response turns against itself. The autoimmune response is triggered by a combination of genetic and environmental factors. The presence of auto-antibodies to thyroid peroxidase is an indication of autoimmune thyroid disease. The two most common thyroid autoimmune diseases are Graves disease and Hashimoto thyroiditis, abnormal levels of thyroid hormones and an enlarged thyroid gland (goiter) are features of these disorders.

Autoantibodies to thyroid peroxidase are present in about 75 percent of people with Graves disease and 90 percent of those with Hashimoto thyroiditis [30-34]. Thyroid peroxidase antibody positivity is seen in 10–15% of the general population and is not an indication for treatment where there is no biochemical abnormality of thyroid function. The thyroid peroxidase are important for ruling out autoimmune thyroid diseases, the normal range of a anti-TPO as recommended by Aeskulisa kit is ≤ 40 IU/ ml. In this report we described only 6 patients 11.76 % (6 out of 51) upper than 40 IU/ ml from the total but the more with less, as mentioned in Table-3, whom developed a symptomatic

autoimmune thyroid peroxidase through exposure periods for gleevec treatment represented by (8 months, 1.5, 3, 4, 7 and 9 years) respectively which they taken 400 mg dose of a drug and their aged were (30y F, 33y F, 46y F, 53y F, 42y M, 50y M). The first of the forth cases revealed by females, while the rest revealed by male and these results corresponded with previous studies [35, 36, 37].

Table 3- Distribution of patients study according of Cut off

Group (Cut off)	No.	Percentage (%)	Chi-square (P-value)
Less than 40 IU/ml	45	88.24	14.051 ** (0.00163)
More than 40 IU/ml	6	11.76	
** (P<0.01).			

Thyroid biopsies were not performed to verify the diagnosis because the patterns of thyroid auto-antibodies were characteristic. These observations raised several questions as to whether thyroid circulating hormone levels and autoimmunity screening tests should be performed before starting long-term treatment with a gleevec, and questions regarding the management of overt thyroid diseases during the treatment itself and the exacerbation of aut-oimmune diseases during gleveec therapy [36, 37]. The incidence of thyroid diseases varied and the time between start of therapy and development of thyroid disease varied considerably with intervals ranging [38]. The data suggest that at least the presence of anti-thyroid peroxidase must be carefully documented in patients undergoing a gleevec therapy, and that possibly, something other than gleevec should be employed if proof of thyroid autoimmunity is found. On the other hand, knowledge of the underlying hematological disease and the availability of different drugs active against it play a central role in the therapeutic strategy. Regarding the management of overt thyroid diseases during long-term a-gleveec treatment, a distinction must be made between glandular hyper/or hypofunction. Interlukins and interferons are used for treating CML and other conditions increase antibodies that put patient at risk factor hypo /or hyperthyroidism, so as some drugs used in cancer chemotherapy such as sunitinib (Sunet) or imatinib (gleveec), can also cause thyroid dysfunction.[27].

The mechanism by which gleevec induces autoimmune thyroid peroxidase is unclear. A crucial step seems to be the induction of HLA class I expression on the surface of thyroid cells. The expression of MHC molecules on cell surfaces,in association with normal cellular antigens, might be sufficient to break tollerance and induce autoantibody formation and activation of cytotoxic or suppressor T lymphocytes and NK cells. Hyperthyroidsm and goiter in Graves'disease are caused by thyroid-stimulating autoantibodies that bind to and activate the thyroid- stimulating hormone (TSH) receptors on thyroid follicular cells. And hypothyroidisim by thyroid-cell death due to an accumulation of lymphocytes, predominantly T cells, in the thyroid [38]. Large granular lymphocytic leukemia is frequently accompanied by autoimmune processes such as rheumatoid arthritis often manifested as (Felty's syndrome) and immune-mediated cytopenias [39]. T-cell large granular lymphocytic leukemia was initially described as a clonal disorder of large granular lymphocytes involving blood, bone marrow, spleen, and liver [40]. This disorder is characterized by the presence of abnormal to activated effector cytotoxic T lymphocytes (CTLs) [41,42]. Large granular lymphocytes can secrete several cytokines that may play a role in immune-mediated cytopenias and autoimmune disorders [42].

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