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Detection of Autoimmune Antibody Among Some Chronic Lymphocytic Leukemia Sudanese Patients - Khartoum, Sudan (2008)

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Abstract

Introduction: Few data are available concerning the prevalence of autoimmune disease in chronic lymphocytic leukemia patients at diagnosis as well as at clinical outcome. This study was carried out in the radioisotope center in Khartoum - department of blood bank from the period 2 August to 20 September 2008.

Aim: The aim of this study is to detect the presence of auto-immune antibody among chronic lymphocytic leukemia patients.

Method: Two ml of venous blood were collected from 50 CLL patients of both sexes and different ages. All specimens were tested for autoimmune antibody by using direct Coomb's by the immune diffusion gel technique. A structured pre tested questionnaire was used to collect the qualitative data

Results Analysis showed that 38% of the patients have autoimmune antibody in their serum. Also it confirmed that there is significant difference between the presence of autoimmune antibody, jobs (0.004), the duration of the disease (0.019) and there is no significant difference between the presence of autoimmune antibody and age (0.051) as well as the sex of the patients (0.960).

Recommendations: CLL patient must be investigated for autoanibodies, which can help in improving management and enhance controlling the prognosis of the patient.

الخلاصة

مقدمة: البيانات المتوفره قليلة بخصوص انتشار الاضداد منيعة الذات المتعلقة بمرض ابيضاض الدم الليمفاوي المزمن سواء أكانت عند التشخيص او في المخرجات السريرية. وقد أجريت هذه الدراسة في مركز النظائر المشعة بالخرطوم في قسم بنك الدم في الفترة من 2أغسطس الي 20سبتمبر 2008.

الهدف: الهدف من هذه الدراسة هو الكشف عن الاضداد منيعة الذات بين مرضى ابيضاض الدم الليمفاوي المزمن.

الطريقة: تم جمع 2مل من الدم الوريدي لعدد 50 مريض من الجنسين و من مختلف الأعمار. تم الكشف عن الاضداد منيعة الذات في كل العينات باستخدام اختبار كومبس المباشر وبتقنية الانتشار المناعي في الجل. وتم استخدام استبيان (مختبر مسبقا) لجمع البيانات النوعية.

النتائج: أظهرت نتائج التحليل أن 38٪ من مرضى ابيضاض الدم الليمفاوي المزمن لديهم اضداد منيعة الذات. وأكدت النتائج أيضا وجود فرق معنوي عند مقارنة ظهور الاضداد منيعة الذات و نوع الوظيفة (0.004)، ومدة المرض (0.019) وليس هناك فرق معنوي بين ظهور الاضداد والعمر (0.051)، وكذلك جنس المرض(0.960).

التوصيات: يجب الكشف عن الاجسام المضادة لدي كل مرضي ابيضاض الدم اليمفاوي المزمن ، حيث انه يمكن أن يساعد في المعالجة و السيطرة على تطور المرض وتحسين مستقبله.

Key words: Chronic lymphocytic leukemia, autoimmune antibody, direct Coomb's, immune diffusion gel technique

Introduction:

Chronic lymphocytic leukemia (CLL) is a monoclonal disorder characterized by a progressive

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accumulation of functionally incompetent lymphocytes and affects mostly the B lymphocytes and causes suppression of the immune system, failure of the bone marrow and infiltration of malignant cells into organs. Although chronic lymphocytic leukemia starts in the bone marrow it can spread to the blood, lymph nodes, spleen, liver, central nervous system (CNS), and other organs as a result of a prolonged lifespan with impaired apoptosis, it does not usually form a solid mass or tumor.(1)

Chronic lymphocytic leukemia (CLL) is most common in the Western world but rare in the Far East (e.g. China, Japan), where it is estimated to comprise 10% of all leukemias.(2)

In contrast to other forms of leukaemias there is no incidence induced by previous chemotherapy or radiotherapy. There is a seven-fold increased risk of CLL in the close relatives of patients. It has a peak incidence between 60 and 80 years of age and more common in males. The etiology is unknown but there are geographical variations in incidence.(2)

The link between autoimmunity and chronic lymphocytic leukemia may result from a common aetiological origin (genetic, hormonal, or environmental factors) or alternatively, from paraneoplastic syndromes. A large number of auto antibodies have been identified, but little is known about their clinical significance. The commonest laboratory findings are: anemia secondary to bone marrow involvement with CLL, splenic sequestration of red blood cells, autoimmune hemolytic anemia and pure RBCs aplasia and thrombocytopenia due to splenic sequestration and auto-immune thrombocytopenia.(3)

The autoimmunity in B-CLL is observed much more commonly in patients treated with fludarabine, which is known to suppress the circulating $CD4^+$ T cells. (4)

A striking feature of autoimmunity in CLL is that it appears to be directed against constituents of the blood with no documented propensity to other targets. However, despite the importance of this immune deregulations in CLL, the causes of autoantibody production remain unclear.(3)

Some recent researches were performed to determine whether these auto antibodies are the products of the neoplastic B-CLL clones. Immunoglobulins (Ig) were eluted from washed red blood cells (RBCs) obtained from two CLL patients at the time they had autoimmune (DAT-direct antiglobulin test-positive) hemolytic anemia. The light chain phenotypes of these eluted auto antibodies were determined and found to be monotypic with exact correlation to the light chain expressed on the surface of the B-CLL clones. The eluted antibodies from RBC of DAT negative patients or normal volunteers failed to demonstrate measurable amounts of Ig. In contrast, Ig eluted from RBCs obtained from SLE patients with DAT positive hemolytic anemia was found to be polyclonal auto antibodies exhibiting both light chain types. Furthermore, CD5+B lymphocytes obtained from the same above mentioned two CLL patients (DAT+) produced, in vitro under stimulation with phorbal myristate acetate (PMA), monoclonal antibodies which react and bind to RBC. Thus these studies provide direct evidence demonstrating that the antibodies causing the autoimmune hemolytic anemia in the two CLL patients are the products of the B-CLL obtained from neoplastic clones. (5)

The main objective of this study is to detect the auto immune antibody among chronic lymphocytic leukemia Sudanese patients, also it aimed to determine if there is a relationship between the presence of autoimmune antibody and some factors like age, sex, job of the patients as well as the duration of this disease.

Material and method

This study was carried out in the radioisotope center of Khartoum, Sudan during the period from 2 August

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to 20 September, 2008. A total of 50 venous blood samples were taken (using clean venopuncture technique) from differently aged CLL Sudanese patients from both sexes. A structured pre tested questionnaire was used to collect the qualitative data. A written informed consent was obtained from all research subjects after the explanation of the research objectives, and any refusal was respected and the confidentiality of the data was ensured.

Antibodies detection was done by immuno diffusion gel technique "The DiaClon Rh-subgroups" micro-card was used according to the standerd guidelines. Known positive and negative controls were included in accordance with the relevant guidelines of quality assurance.[6]

The results were interpreted as follows:

Positive: agglutinated cells forming a discrete red line (++++) on the surface of the gel or agglutinates dispersed in gel (+++; ++; +) depending on the size and position of the agglutinates in the gel. Negative: Compact button of red cells in the bottom of the micro-tube (Figure 1).

Results

A total of 50 CLL patients participated in this study, 68% (n=34) of them were males and the remaining 32% (n=16) were females. Majority of them were from Western Sudan (59.5%): Darfour 33.3% and Kordofan 26.2%) (Figure 2).

The antibody screening test (direct Coombs test) result showed that 38% (n=19) of the study population had autoimmune antibodies (Table 1).

According to the job distribution the result showed that all the females were housewives and 18 (52%) of the males had outdoor jobs (farmer) and the remaining have indoor jobs; such as office work and others (Table 2).

Regarding the presence of autoatibodies, the study also showed that there was a highly significant difference between the appearance of the autoantibodies and the type of the jobs (0.004) and the duration of the disease (0.019), but there were no significant differences according to age or gender (Tables 2 and 3).

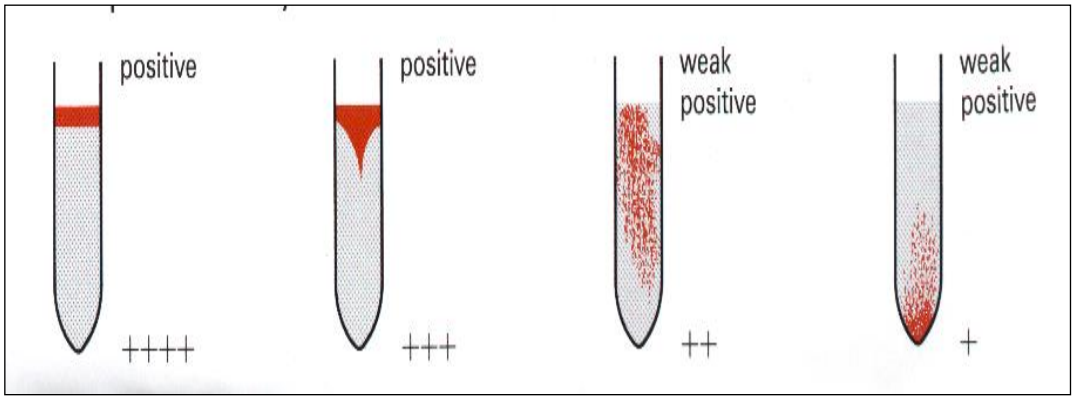

Figure 1: Gel test

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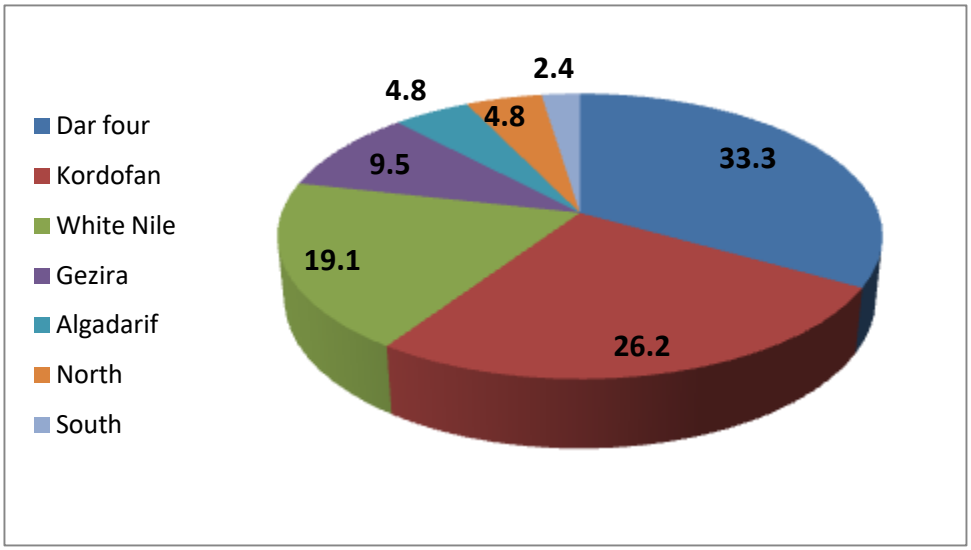

Figure 2: Frequency of residence among study population

Table 1: Frequency of positive & negative antibody screening test.

Antibody screening test	Frequency	Percent
Positive	19 (6 females\13males)	38%
Negative	31(10 females\ 21males)	62%

Table 2: Frequency and significance of autoantibody according to occupations.

	Positive	negative	Chi-square	P.value
Farmer	**11**	**7**		
Not farmer	**2**	**14**	**8.476**	**0.004**

Table (3) Frequency and significance of autoantibody according to duration of the disease (in months).

Duration	Positive	negative	Chi-square	P.value
3 -9	**3**	**15**		

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10-16	**9**	**13**	**7.902**	**0.019**
17-23	**7**	**3**		

Discussion:

Gender distribution among the study population shows higher incidence of CLL in males than females with a ratio of 1.7: 1. This result is similar to that of Matutes E, Polliak A [The incidence of CLL is higher in males than in females, with a male-to-female ratio of 1.7:1. (1)

Frequency of residence (or tribe) among study population found increased CLL in western Sudanese patient especially those from Darfour and Kourdofan.This may be due to their life style or some environmental factors which have to be studied.

Thirty eight percent of the study population have autoimmune antibodies in their serum with positive direct Coomb's test and the others 62 % were negative, similar to that previously reported by Rear **(7)** who found that 30% of patients developed autoimmune hemolytic anemia (AIHA). In contrast Katrina Vanura (8) found that 22(11.8%) CLL patients of 186 patients suffered from autoimmune antibodies. Clinically, Barcellini (9) found 16% of CLL patients to suffer from autoimmune other than disease stage, no clinical or prognostic data were included in this study. Fifty-two cases of (AIHA) were observed within a series of 1203 patients (4.3%) with (CLL) followed at a single institution as reported by Francesca (10). These different results may be due to genetic factors or sample size or may be due to the different methods used to test for the autoantibodies.

The incidence of CLL in farmers is higher than others occupations (52 %.of all) with a P valu <.004 which may be due to the use of chemical substances in the agriculture processes. In spite of increased positive results in males (68 %) more than females (32%) the statistical analysis showed that there is no significant difference according to gender (P= 0.9).

Thirty one cases of the total of 50 are above 50year and have autoimmune antibodies with a significant difference with other age groups. According to Francesca (10) study which is carried out on the entire series of 1203 patients, age (*P* = .01), and gender (*P* < .01) emerged as independent factors significantly related to the occurrence of DAT-positive anemia (AIHA) at the time of CLL diagnosis., older age, and male gender were significantly linked with an increased rate of IAHA at CLL diagnosis.

At multivariate analysis, gender and age emerged as independent factors significantly correlated with the occurrence of AIHA. Men and aged patients showed a significantly higher rate of AIHA. Although there is no explanation for the higher rate of AIHA among men, the higher number of AIHA among elderly patients is not surprising **(11).** It is possible that, an impaired function of the aged thymus may lead to an imbalance among autoregulatory $CD4^+$ and autoreactive T-lymphocyte subsets that may predispose to autoimmunity. (12)

According to the duration of disease 10 cases (20%) of all patients have a duration more than 17 months, 7 of them were positive to direct Comb's test , the analysis confirmed that there is significance relation(P< 0.019) between the duration of disease and the appearance AIHA .

In conclusion, this study indicates that an autoimmune antibody is a common event in CLL with a

significantly higher incidence in older male patients.

Conclusions and recommendations:

An autoimmune antibody is a common event in CLL with a significantly higher incidence in outdoor workers (farmers) and in patients who have disease for a long duration. Autoantibody screening test must be used as routine test for CLL patients, which can help in improving their management and controlling the prognosis of the patient.

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