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CD23 and CD6 Molecule Expression in Chronic Lymphoid Leukemia (CLL) Pateints

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Abstract :

This study has been conducted on blood samples from patients with chronic lymphoid leukemia's (CLL) who were diagnosed and treated at The National Center of Hematology between January 2006 to September 2009.

The diagnosis was established depending on clinical and hematological findings. Immunophenotyping was performed by indirect immunofluorescence technique using fluorescent microscopy to identify CD 23 and CD 6 markers expression on peripheral blood mononuclear cells. Positive cell is seen with multiple fluorescence dyes around the membrane or by bright green membrane fluorescence, the cases considered as positive for marker when the marker is expressed in ≤ 30 of cells.

Sixty cases were newly diagnosed untreated CLL patients with mean age of 64.5 ± 10.1 years with 9.4% of cases below the age of 45 years and male to female ration of 3:1were involved in this study.

Immunological study showed that all the cases were CD 23 positive while CD6 were positive in 45 (75%) out of 60 cases and negative in 15(25%) out of 60 cases. These percentages in all CD 23 positive cases and CD6 positive CLL patients were higher significant than normal group (p=0.000).

In conclusion, the positive CD23 expression clarify that the majority of chronic lymphoid leukemia are B-cell type .B-cell is the most common which is heterogeneous disease regarding clinical presentation ,hematological findings and morphological feature although CD6 is not specific marker for diagnosis of CLL ,but it may help in the diagnosis of CLL.

Key words: *CD23 & CD6 markers; CLL; Immunophenotyping; Immunofluorescence.*

Introduction:

Chronic lymphoproliferative disorders (CLDPs) are heterogeneous complex group of diseases ,derived from neoplastic clonal proliferation of cytologically and immunophenoltypically mature B or T cells (1,2,3) the disorders that predominantly affect lymph nodes and other extramedullary sites are generally regarded as lymphoma whereas disease with predominant bone marrow and blood manifestation are generally labeled as leukemia(1) .

With development of immunological techniques for detection of surface membrane markers it has become apparent that majority of CLPDs are of B cell origin(4) among them one disorder B- chronic lymphocytic leukemia(B-CLL) account for at least 85% of leukemic CLPPs (1,4,5) ,60% of all CLPDs and 25-30% of all leukemia's (2,6,7).

Chronic lymphoid leukemic of T-cell origin have been reported to account for less than 5% of total number of CLPDs the lymphocytes consist of heterogeneous population of cells that differ greatly from each other in term of origin lifespan preferred areas of settlement with in lymphoid organs surface structure and function .

The most precise and quantitative method providing a clue of lymphoid lineage and function is based on identification of certain proteins or glycoproteins either intracellular or on cell surface membrane that called cluster of differentiation marker (CD marker) (8, 9).

Monoclonal antibodies resulting from the hybridomas biotechnology has led to the wide availability of antibodies suitable for typing leukemic cells (10).The character

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istic feature of B-CLL is the positivity for CD5 and CD 6 expression on B- cells is not specific for B-CLL because these markers also found in some other B-CLL malignancies (11,12,13). Most cases of B-CLL are positive for the CD23 antigen which is not present on the majority of other type of CLL malignancies (8, 19).

Absence of CD23 antigen or a weak expression of this antigen together with positivity for FMC7 is associated with a poor progress (19).

In these leukemia, there is often a high expression of surface IgM, which suggests an intermediate stage between B-CLL and B-Cell prolymphocytic leukemia (B-PLL) (8).

Material and methods:

Patients:

Between January 2006 to September 2009, sixty cases with chronic lymphoid leukemia's were selected for this study. All cases were diagnosed and treated in National Center of Hematology. The diagnosis was established depending on clinical and hematological findings.

Control group:

Thirty healthy adults (males and females) with age range (30 – 65(year were evaluated clinically and hematologically.

Sampling:

A total of 5 ml of EDTA blood sample were collected from each patients and control for total blood count and immunophenotyping study(14).

Immunophenotyping:

This was performed on blood samples of all cases immediately after sample collection. Immunophenotyping was performed by indirect immunofluorescence technique using CD23 and CD6 markers on isolated B-cell.

Reagents:

Normal saline 9 g/l NaCl; RPMI 1640 media (Brown Deer, W1; USA)

; RPMI -5% HI-FCS: RPMI 1640 tissue culture media with heat inactivated fetal calf serum (NYCOMED, AS; OSLO).; Ficoll-Hypaque separation fluid: Lymphoprep (PHARMACIA;SWEDEN, specific density 1.077);Phosphate buffered saline with 1% bovine serum albumin ,0.05% sodium azide and 2% AB serum (PBS-BSA-AZIDE-AB-buffer) of pH(7.4) (FLOW LAB. LIMITED. SCOTLAND); Scrabbled nylon wool (FLOW LAB. LIMITED. SCOTLAND); Mouse antihuman CD23: FITC (SEROTIC; UK).; Mouse antihuman CD6: FITC (SEROTIC;UK).; Mounting media prepared by mixing equal volume of glycerol and phosphate buffered saline.

Procedure:

1. Isolation of peripheral blood lymphocytes (3,15).

Peripheral blood were collected in anticoagulant (EDTA) and mononuclear cells are obtained by Ficoll-Hypaque density centrifugation. The cells are washed twice in phosphate buffered saline (PBS) and resuspended in PBS-BSA-AZIDE- AB-buffer.

2. Separation of B- lymphocytes(16).

The mononuclear cells were resuspend in warmed (37oC) RPMI- 5% HI-FCS and passed through nylon(0.1g) nylon wool packed columns which were filled with RPM1- 5% HI-FCS above the nylon wool and incubated at 37oC for 30 minutes,allowing the B-lymphocytes to adhere to fibers. The non adherent cells (T- cells and some platelets) were flushed out and the remaining adherent cells and few platelets were removed from the column after twice elution by adding 1.5 mL of cool RPM1- 5% HI-FCS and mechanical agitation of the wool and then collected in clear container and the recovered cells were washed twice with (RPMI-5% HI-FCS).

3. Indirect immunofluorescence staining

- Fifty µL of cell suspension (106 cells) were incubated with adequate volume (5- 50µL) of optimally diluted labeled monoclonal at 4 o C.
- The mixture was washed twice in PBS-BSA-AZID-Ab buffer.
- Centrifugation at 400g for 5 minute was performed and supernatant was discarded
- The cells were resuspended in 0.2 ml of mounting media.
- Drop of the suspended cells was put on slide gently and was covered by cover slide and air bubble was avoided.
- The result was read under fluorescent microscopy using FITC (fluorescein isothiocyanate) filter set.
- Negative control was performed using IgG1; FITC instead of McAB.

4. Evaluation of immuneophenotyping results

At least 200 cells should be analyzed for the fluorescence stain ; a positive cells in seen with multiple fluorescence dots around the membrane or by bright green membrane fluorescence (3,17) a cutoff point of 30%of cell stained with CD23 and CD6 markers was considered to be positive(3,18).

Results:

In the sixty cases of chronic lymphoid leukemia's included in this study had an age range from 45-84 years .The median age was 64.5 years and mean age was 62 years (table1)

They included 45 males and 15 female with male to female ration (3:1).These disorders included 60 CLL cases all of them were newly diagnosed untreated patients.

Immunophenotyping study showed that all cases were CD23 Positive while CD6 were positive in 76.6% out of 60 cases (table2).

On comparing CD6+ CLL with CD6 CLL with regard to sex and age, There was no significant difference between the two groups (table 3& 4).

Table (1) age and sex distribution in CLL cases

Age(years)				Sex		
Median	Mean	SD*	Range	male	Female	Male: female
62	64.5	9.65	45-84	45/60	15/60	3:1

**Standard deviation*

Table (2) immunological data of CLL cases

Marker	Normal control n=30	Patients CLL n=60
CD 23	15%	All cases positive
CD 6	12%	76.6%

Significant difference between the CLL patients and healthy control (p=0.000)

Table (3) comparison between CD6- and CD 6+ CLL according to sex

Sex	CD 6^- CLL	$CD6^+$ CLL	T OTAL
Female	6 (40%)	9(20%)	15(25%)
Male	9(60%)	36(80%)	45(75%)
Total	15(100%)	45(100%)	60(100%)

Table (4) comparison between CD6- and CD 6+ CLL according to age

Numbers of cases	Age(years)			
	Range	Median	Mean	Standard deviation
CD 6^+ CLL (45)	45-65	59	60.9	10.09
CD 6^- CLL (15)	48-84	60.5	59	5.45
Total (60)	45-84	60.46	61	9.28

Discussion:

The current study attempted to identify the CD23 and CD6 molecule expression in chronic lymphoid leukemia cases by using indirect immunofluorescence technique. All the patients samples expressed CD 23 (out of point is ≥ 30% of positive cells) (3, 18) which was significant higher than normal control group (p=0.000).

The positive CD23 in all CLL proved that all the patients CLL were of B-Cell origin, and this result was in agreement with other studies on CD23 which revealed that most cases of B-Cell are positive for CD23 antigen, which is not present in the majority of other types of B-cell malignancies (8,23).Positive CD6 expression 75% of CLL cases (cut off point is ≥ 30% of positive cells (3,18).The percentage of CD6 positive B-cells was 75% was significant higher than normal control group (12%) (p=0.000).

Another studies also noted that CD6 expression on B-cells is not specific for B-CLL because CD6 is also found in some other B-cell malignancies (11, 23, 1).

Many studies all over the world have focused on immunophenoltyping of hematological malignancies using different technique.

B-cell chronic lymphocytic leukemia (CLL) accounts for approximately 30% of reported leukemia cases in western countries. CLL regard the most frequent in western hemisphere (4) are rare in Chinese and related race (19).

It has an incidence of 2.3-3.3 per 100,000 people.Even though the disease more frequently affects elderly people (median age 60 years), a significant fraction of cases (10-15%) is diagnosed in subjects younger than 50 years old (20).

The median age of 62 years similar to other studies such as Badrossian revealed similar median age 60 years and male to female ratio of 2:1 (21). While AL- Rubaie, H.A on 140 CLL cases revealed similar median age and male: female ratio of 2:4(17). .Also in Iraq Mahdi , S.M. show median age 58 years and male to female ration of 2.1: 1(22).

Western studies also described the same age and sex distribution (2, 5).

In this study there were no CLL cases below the age of 40 years also the rare of CLL cases below the age of 40 years like in many studies (5).

Conclusions:

-The positive CD23 expression clarify that the majority of chronic lymphoid leukemia's

are of B-CLL type, while T- chronic lymphoid leukemia's are extremely rare.

-CD6 is not specific marker for diagnosis of CLL, but it may help in the diagnosis of CLL.

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ظهور علامتي العنقود التفريقي 23 و 6 عند مرضى ابيضاض الدم اللمفاوي المزمن

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الخلاصة:

أجريت هذه الدراسة من كانون الثاني عام 2006 الى ايلول عام 2009 حيث تم فحص (60) عينة دم من مرضى مصابين بابيضاض الدم اللمفاوي المزمن بعد ان تم تشخيصهم في المركز الوطني لبحوث وعلاج امراض الدم ومن ثم علاجهم حيث اعتمد تشخيص هذه الحالات على العلامات السريرية والفحوصات الدموية الخاصة بالاضافة الى المسحات الدموية من قبل اختصاصين في امراض الدم في المركز ولقد تم التشخيص بطريقة الالق المناعي باستخدام الجهر الالقي (الفلورسنت) لاختبار النمط المناعي الظاهري . لقد هدفت الدراسة الى التحري عن ظهور علامتي العنقود التفريقي (23)و(6) في خلايا الدم المحيطي احاديه النواة لمرض ابيضاضات الدم المزمن حيث تم اجراء اختبار النمط المناعي الظاهري لجميع الحالاتبواسطة تقنية الالق المناعي غير المباشر باستخدام الجهر الالقي حيث ظهر التفاعل الايجابي للخلايا اللمفاوية مع العلامة المناعيه بشكل الق اخضر على غشاء الخلايا اللمفاوية بشكل يفوق 30% من مجموع الخلايا .

لقد شملت هذه الدراسة (60) حالة مرضية مشخصة حديثاً وغير معالجة معدل اعمارهم (±64.5 10.1) سنة وبنسبة 9.4 % من الحالات كانت بعمر اقل من 50 سنة وليس هناك حالة اقل من 45 سنة ونسبة الذكور المصابين للاناث 3:1 .

اظهرت الدراسة المناعية ظهور علامة العنقود التفريقي (23) موجب في كل الحالات المرضية في حين كانت علامة العنقود التفريقي (6) موجودة في45 حالة أي بنسبة (75%) من اصل 60 حالة والنسبة السالبة هي (25%) أي 15 حالة من اصل 60.وهذه النتائج هي اعلى مما هو في معدلات مجموعه السيطره بشكل محسوس احصائيا تحت مستوى احتمال (p=0.00) .

ونستنتج من هذه النتائج ان ظهور العلامة(23) في كل الحالات يعني ان الحالات المفحوصة المصابة سرطان الخلايا اللمفاوية المزمن هي نوع الخلايا اللمفاوية (B) ولكن ظهور العلامة(6) هو غير متخصص في تشخيص هذه الاسقام ولكنه يساعد في التشخيص.