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Immunophenotyic profile of adult acute lymphoblastic leukaemia in Iraq, a one year experience

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Abstract:

Background: Acute lymphoblastic leukaemia accounts for approximately 14% of leukaemia cases in the adults with B linage subtype being more common than T linage subtype. immnophenotyping using the multicolour flow cytometry is an essential tool for diagnosis, classification as well as treatment guidance and prognosis.
Objectives: This study aims to provide an overview of the immunophenotypic profile of adult ALL cases in Iraq, it's possible link with other characters and also study the frequency of expression of aberrant myeloid antigens in them.
Patients and methods: this is a descriptive study included 64 adult patients diagnosed as ALL in haematology unit at Baghdad teaching hospital for the period between May 2014 till May 2015 , immunophenotyping was carried out at the flow cytometry laboratory in the nursing home hospital using 6 coloured (BD-FACS-Canto II System from Becton Dickenson).
Results: of the study population 63.7% of the cases were B-ALL and 27.5% were T-ALL, there was no significant difference in respect to age and gender between them. Of the CD markers used both CD10 and CD34 were significantly associated with B-ALL compared to T-ALL (p values 0.002 and 0.003 respectively).aberrant myeloid Ag expression was seen in 43.18% of B-ALL cases and in 52.6% of T-ALL cases with no significant difference however aberrant myeloid Ag expression was significantly associated with CD34 expression with a p value of 00.018.
Conclusion: Immunophenotyping retains a crucial place in the work up for patients with acute lymphoblastic leukaemia. CD34 was significantly associated with B linage ALL and with aberrant myeloid Ag expression, larger study with clinical evaluation and cytogenetic study is recommended to evaluate the impact of those findings over prognosis.

Key words: *immunophenotying, B-ALL, T-ALL, multichannel flow cytometry, aberrant myeloid Ag expression.*

Introduction:

Acute lymphoblastic leukaemia is a common neoplasm of haemopoietic precursors that occur with a higher frequency in children than in adults. In adults ALL accounts approximately for 14% of leukaemia cases with an incidence estimated to be less than 1 % (1). In the early classification schemes the classification was based mainly on cytomorphology supplemented by immunohistochemistry (FAB classification). The recent WHO classification emphasizes the use of immunophenotyping for accurate categorization of ALL cases and abandons the use of FAB classification as it has no prognostic or therapeutic implications. In the WHO classification precursor lymphoid neoplasms are classified into precursor B lymphoblastic leukaemia/lymphoma and to precursor T lymphoblastic leukaemia/lymphoma (2). Immunophenotyping by means of multi-channel flow cytometry (MFC) has become the standard procedure for ALL diagnosis and subclassification, and was also developed as useful tool for the detection and monitoring of minimal residual disease (3).Flow cytometry can identify several cellular properties, including size, cytoplasmic and nuclear complexity. It can provide correlated data that links different population profiles all based on single cell analysis. (4)
In Iraq immunophenotyping has only been recently introduced to the panel of investigations for ALL. The aim of this study is to provide an overview of the immunophenotypic profile of adult ALL cases in Iraq and also study the frequency of expression of aberrant myeloid antigens in them.

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Patients and methods:

A total of 64 adult (age more than 18 years) untreated newly diagnosed ALL patients admitted to the haematology unit at Baghdad teaching hospital for the period between May 2014 till May 2015 were included in this study, informed consent was taken from the study population .ALL Diagnosis was based on morphology and immunophenotyping.
immunophenotyping was carried out at the flow cytometry laboratory in the nursing home hospital using 6 coloured (BD-FACS-Canto II System from Becton Dickenson). Either peripheral blood or bone marrow samples were used according to the availability and blast percentage. Blood or the bone marrow samples were collected in EDTA tubes and processed within 24 hours of collection a panel of fluorochrome-conjugated monoclonal antibodies were used, all the antibodies were obtained from Becton Dickenson (USA). Fluorochromes used were fluorescein isothiocyanate (FITC), phycoerythrin (PE) or peridinin chlorophyll protein (PerCP), and were directed to the following antigens CD1a, CD2, cytoplasmic (c) CD3, CD4, CD5, CD7 and CD8 ,CD10, CD19, CD20 and cCD79a ,CD13, CD33, CD117 , CD11b, cytoplasmic myeloperoxidase (MPO)], CD14, CD15 CD64 CD34, CD38 ,human leukocyte antigen-DR (HLA-DR)] ,CD56 and cytoplasmic TdT .(5)

Data acquisition and sample analysis was performed in a BD FACS CantoII (6- color, Becton Dickinson, USA), using FACSCANTO Diva (Becton Dickinson), after calibration with the Calibrate bead kit (Becton Dickinson).The blast gating strategy included using dot plots of CD45 expression versus intracellular complexity (side scatter angle, SSC) .A total of 10,000 events were acquired in the target gate. Antigen was considered as positive if 20% or more of the blast cells reacted with a particular antibody.
Statistical analysis was done using SPSS 20, Chi square test was used for categorical variables , and student t test was used for continuous variables values less than 0.005 were considered as significant.

Results:

Patient's characteristics: in this descriptive study 64 adult patients diagnosed with ALL during the study period were included in the study , 40 males with a mean age of 30.46± 13.18 and 24 females with a mean age of 35.21± 15.24, there was no statistically significant difference for the age between males and females. The majority of the patients were less than 25 years old (16 of B-ALL and 7 of T-ALL) with only one case of B-ALL was older than 65 years, all the results are summarized in figure 1

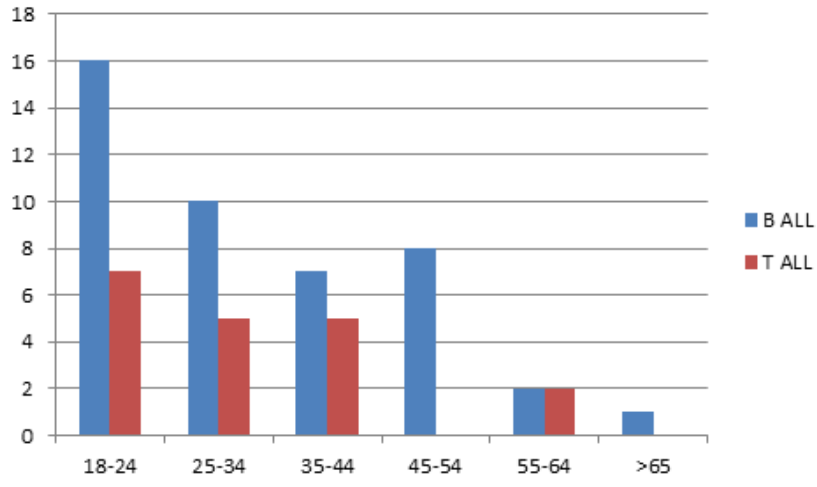

Figure (1): age distribution of the study population.

Immunophenotypic charecteristics of ALL subtypes:
According to immunophenotying results, 44 of the cases were B linage -ALL, 19 cases were T linage -ALL and one case was diagnosed as Blastic plasmacytoid dendritic cell neoplasia . From the B linage ALL 39 of the cases showed CD10expression and considered as common ALL and only 5 cases were CD10 negative. The expression of the various CD markers in ALL subtypes is summarized in table 1.
CD10 and CD34 expressions were associated with B-ALL rather than T-ALL as shown by Chi square test with a p value of 0.002 and 0.003 respectively. On the other hand HLA-DR expression was seen in all the B Linage cases while only 6 cases of T linage ALL were positive for this marker with a highly significant difference(p value less than 0.001) .As expected markers that are specifically associated with B-ALL (19,20,79a) or with T-ALL (CD2,CD3,CD4,CD5,CD7,CD8) were highly significantly different between the two types of leukaemia.

Table (1): the expression of various CD markers in ALL subtypes.

CD markers positive	(B- ALL (44)(63.7%		(T- ALL (19)(27.5%	
	Number	(%)Percentage	Number	(%)Percentage
CD 34	36	81.8%	8	42.1%
HLA-DR	44	100%	6	31.5%
CD38	29	65.9%	16	84.2%
CD1a	0	0%	1	5.2%
CD2	1	2.27%	8	42.1%
cCD3	0	0%	18	94.7%
CD4	1	2.27%	11	57.8%
CD5	0	0%	14	73.6%
CD7	0	0%	19	100%
CD8	0	0%	10	52.6%
CD10	39	88.6%	10	52.6%
CD19	42	95.4%	0	0%15.7%
CD20	28	63.6	0	0%
CD79a	37	84.09%	3	15.7%
CD11b	3	1.3%	1	5.2%
CD33	11	25%	6	31.5%
CD13	12	27.2%	3	15.7%
CD14	0	0%	0	0%
CD15	0	0%	0	0%
CD16	3	6.8%	1	5.2%
CD64	0	0%	0	0%
CD117	0	0%	3	15.7%
cTdT	41	93.1%	16	84.2%
cMPO	0	0%	0	0%
CD56	2	4.5%	1	5.2%

Of the common B linage CD markers CD19 was the most common present in about 95.4% of the B-ALL cases followed by cTDT (93.1%), the frequency of expression of B linage CD markers in B-ALL is illustrated in figure 2

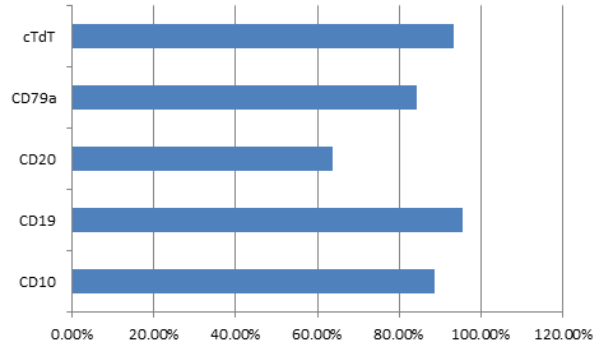

Figure (2): the frequency of expression of B linage CD markers in B-ALL:-

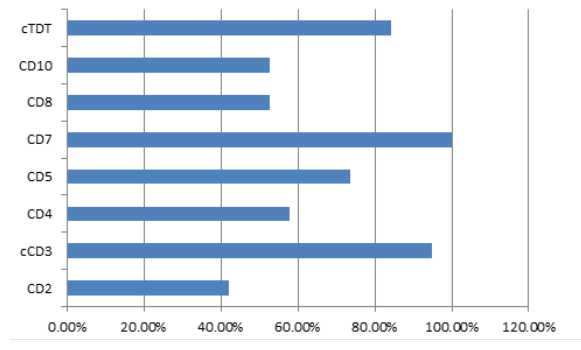

Figure (3): the frequency of expression of T linage CD markers in T-ALL:-

For T linage ALL the commonest CD marker was CD7 that was expressed in 100% of the cases followed by cytoplasmic CD3 all these findings are summerized in figure (3)

Aberrant myeloid Ag expression in ALL subtypes:

Out of the 64 cases included in the study aberrant expression of CD13, CD33,CD16 and CD 11b was seen in 19 cases of B-ALL (43.18%) and in 10 cases of T-ALL (52.6%) , double expression of CD13 and CD33 was seen in 6 cases of B-ALL and only in one case of T- ALL . No significant association was found between ALL subtypes and aberrant Ag expression with a p value of 0.357, all the data are summarized in table 2

Table (2): the frequency of aberrant myeloid Ag expression in different ALL subtypes

Aberrant Ag expression	B-ALL (44)		T-ALL(19)	
	Number	Percentage (%)	Number	Percentage (%)
CD13	12	27.2%	3	15.7%
CD33	11	25%	6	31.5%
CD16	3	6.8%	1	5.2%
CD11b	3	6.8%	1	5.2%
CD13 and CD33	6	13.6%	1	5.2%

However when compared with the other CD markers Aberrant myeloid Ag expression was significantly associated with CD34 expression with a p value of 0.018.

Two cases of B-ALL showed aberrancy for T linage marker, one was expressing CD4 and the other CD2. While none of the T-ALL cases showed B-linage markers expression.

Only one case was diagnosed as Blastic plasmacytoid dendritic cell neoplasia that showed a unique combination of studied .

Discussion:

Immunophenotyping is an essential part of assessment of haematological malignancies and the multicolour flow cytometry can provide useful tool for accurate categorization of these neoplasms providing valuable information regarding the type, subtype as well as treatment guidance and prognosis (5).

In Iraq multicolour flow cytometry has been recently added to the panel of investigations required for assessment of acute leukaemia and this study aims to provide an overall view of the immunophenotypic characteristics of adult ALL cases through the study period that lasted for one year.

In the current study ;The frequency of B-ALL was found to be higher than that of T-ALL (63.7% versus 27.5%) which is quite similar to the frequency demonstrated in most of the other studies(6). The mean age for B-ALL cases was 32.81±14.43 while for T-ALL it was 31.63±13.87 and the difference was not statistically significant. Like most of the other studies the majority of the patients were less than 25 years old for both B-ALL and T-ALL with only one case with B-ALL was older than 65 years. Indeed ALL is a common malignancy in paediatric and adolescence age group (7).

40 of the patients were males and 24 were females with an M:F ratio of 1.6:1 which is slightly higher than the frequency demonstrated in other studies(8,9), this slight male predominance could be due to the sample size or lack of compliance among female patients or possibly due to different population

In the study population B-ALL was seen in about 63.7% of the patients as compared to T-ALL (27.5%) which is a finding quite comparable to other studies like the study of Tong H in China that showed the prevalence of T-ALL among adult patients to be 21.8% (10) and the study of Lahjouji A in Morocco where 30.1 % of adult ALL cases were T-ALL (11)

Of the B linage ALL cases CD10 expression was demonstrated in about 88.6% of the cases that were accordingly categorized as common B-ALL and 11.4% of the cases were negative to that Ag . Surface membrane Immunoglobulin was not included in the Ag panel for patients with leukaemia therefore it was not possible to identify the mature B-ALL subtype. Furthermore, CD10 and CD34 expressions were both significantly higher in B-ALL cases than in T-ALL and many studies have issued the influence of it's expression or it's co-expression with CD34 over treatment outcome and overall survival (12,13,14)

Aberrant myeloid Ag expression was seen in 43.18% of B-ALL cases and in 52.6% of T-ALL cases. The expression of myeloid antigens in acute lymphoblastic leukaemia is quite common with a wide range of incidence as has been reported by many other studies like the study by Adam C (15) where aberrant myeloid Ag was demonstrated in 86.5% of the cases of these antigens CD13 is the most common followed by CD33. In another study myeloid Ag expression was demonstrated in about one third of the cases (16) , not only the incidence of myeloid Ag expression but also it's impact over prognosis has been a subject of debate for many researches .

In the current study a significant association was demonstrated between CD34 expression and aberrant myeloid Ag expression, a finding that has been shown in a previous study by Sharma R (17), the value of this association needs to be further investigated as CD34 expression has been linked with prognosis in many studies (18,19)and whether it's association with myeloid Ag expression is a consistent finding needs a

larger study with clinical evaluation and follow up in order to determine it's impact over treatment response and prognosis.

Conclusions:

Immunophenotyping retains a crucial place in the work up for patients with acute lymphoblastic leukemia .In the current study which examined ALL in adult patients; B-ALL was more frequent with significant association with CD34 expression than T-ALL. Myeloid Ag was expressed in both of them however myeloid Ag expression has been shown to be significantly associated with CD34 expression.

A larger study including larger number of patients with clinical evaluation and if possible cytogenetic study is recommended to evaluate the value of this association over treatment outcome and prognosis.

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دراسة حول الصفات المناعية للخلايا في المرضى البالغين المصابين بسرطان الدم اللمفاوي الحاد في العراق، تجربة سنة واحدة

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الخلاصه:

يمثل سرطان الدم الليمفاوي الحاد حوالي ٪14 من حالات سرطان الدم في البالغين مع نسبة حدوث تقدر بحوالي أقل من ٪1، يعتبر تعيين الخلايا المستند على النمط الظاهري المناعي أداة أساسية لتشخيص وتصنيف، وكذلك التوجيه العلاج

الأهداف: تهدف الدراسة، هذا إلى تقديم لمحة عامة عن النمط الظاهري المناعي لحالات سرطان الدم اللمفاوي الحاد للبالغين في العراق، و امكانية ارتباطه مع الخواص الأخرى، وكذلك دراسة وتيرة التعبير عن مستضدات الدم النخاعي الشاذة في خلاياهم.

طريقة الدراسة: هذه هي دراسة وصفية شملت تشخيص 64 مريضا بالغا مصابين بسرطان الدم اللمفاوي الحاد في وحدة أمراض الدم في مستشفى بغداد التعليمي للفترة ما بين مايو 2014 وحتى مايو 2015، تم تنفيذ فحص النمط الظاهري المناعي للخلايا في مختبر التدفق الخلوي في مستشفى دار التمريض الخاص باستخدام جهاز (BD-FACS-كانتو نظام II من بيكتون ديكنسون) ذو الالوان الستة.

النتائج: من عينة الدراسة كانت ٪63.7 من الحالات B-ALL وكانت ٪27.5 T-ALL، لم يكن هناك اختلاف كبير بينهما فيما يخص السن والجنس. من بين الانماط المناعية الظاهرية المدروسةاظهر العاملان CD10 CD34 ارتباطابشكل كبير مع B-ALL مقارنة مع T-ALL (القيم ف 0.002 و 0.003 على التوالي).كان ظهور الانماط الظاهرية المناعية الشاذةالخاصة بابيضاض الدم النخاعي في ٪43.18 من حالات B-ALL و٪52،6 من حالات T-ALL . مع عدم وجود فرق كبير بينهما و لكن الانماط الظاهرية الشاذة النخاعية كانت مرتبطة بشكل كبير مع CD34.

الاستنتاج: يحتفظ تعيين الخلايا المستند على النمط الظاهري المناعي بمكاناهاما في تشخيص المرضى المصابين بسرطان الدم الليمفاوي الحاد.و تظهر الدراسة ارتباط العامل CD34 مع نوع B-ALL و كذلك مع ظهور الانماط الظاهرية المناعية الشاذةالخاصة بابيضاض الدم النخاعي ،نوصي بدراسة أكبر مع التقييم السريري والدراسة الوراثية الخلوية لتقييم تأثير هذه النتائج على اعلاج و نتائج هذا المرض.