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Haematological, Immunophenotypic, and Cytogenetic profile of Acute Lymphoblastic Leukemia among the Sudanese Population

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Abstract

Leukaemia represents the second type of the ten top most common cancers and the first malignancy of childhood cancer in Khartoum. This study intended to explore the haematological, the immunophenotypic, and the cytogenetic profile of acute lymphocytic leukaemia (ALL) among the Sudanese population. This case-control study included140 patients attending Khartoum Oncology Hospital (KOH), who were diagnosed as ALL and 140 haematological-healthy volunteers between 2016-2018. Data were collected through a purposeful form and from hospital records. The analysis showed that two-thirds of ALL patients were males (63%). The mean age of all subjects was 11 years with a predominance of the rate among the age group 6-10 years of age (34.3%). The B-cell immunophenotype represented 75.5%. Philadelphia chromosome was positive in 4.3% of the ALL children and 30.1% in older age (>18 years) patients. The mean of the complete blood count parameters of ALL patients (Hb=10±1 g/dl, RBC=4±1x 10^6/µl, HCT=34±1%, PLT =269 ±125x10^9/L) was significantly lower than that in controls (Hb=12±1g/dl RBC=5±5.2$10^6$/µl, HCT=36±3%, PLT=353±8x10^9/L)(p =0.00) and the mean of the other parameters (WBC= 19±51 x10^3/µl, MCV=84±12fl, MCH=28±2 pg, Blast=8±21%) was significantly higher (*p*=0.00) than that in controls (WBC= 8±2 x10^3/µl, MCV=81±3fl, MCH=27±1pg, Blast=0.00%). In conclusion, ALL has different features of mean age (6-10 years) and high incidence of Philadelphia chromosome (4.3%) in Sudanese children. While, in keeping with published literature, ALL shows a predominance of males, the B-phenotype frequency rate (%75.5) and the variation in haematological parameters.

Keywords: Malignancy, Leukemia, Anemia, Immunophenotype, Philadelphia chromosome.

مستخلص

يحتل سرطان الدم المرتبة الثانية من عشرة انواع السرطانات الأكثر شيوعا في السودان، ويعتبرسرطان الدم اللمفاوي الحاد عند الاطفال فى المرتبة الاولى من هذه الانواع. الهدف من هذه الدراسة قياس معدلات الدم الكامل، نوعية الظواهر الجينية ونسبتها داخل الخلايا اللمفية البيضاء (BوT). هذه دراسة تحليلية تعتمد على المقارنة بين الحالة والمعيار تم اجراءها في الفترة مابين 2016 الى 2018 تشمل 140 مصاب بسرطان الدم اللمفاوي الحاد بمستشفى الخرطوم للأورام و140معيار غير مصابين بالسرطان . تم جمع المعلومات الخاصة بالمرضي من مكتب الإحصاء والمعمل بهذه المستشفى. أظهرت الدراسة ان ثلثي المرضى من الذكور (63.8%) مقارن ة بالإناث (36.2%). ومتوسط الاعمار 11سنة. واعلى الاعمار اصابة هم ما بين 6- 10 سنة (34.3%) مقارنة بالأطفال من1 – 5سنة (28.6 %) سنة .معظم المصابين يحملون الظاهرة الجينية للخلايا (B) (75.5 %) ونسبة الذين يحملون فيلاديلفيا كرموسوم الايجابي من الاطفال 4.3% ومن الكبار في العمر 30.1%. و قيمة متوسط خضاب الدم (10±1g/dl)، متوسط تعداد كريات الدم ($4\pm1x10^6/\mu$)، متوسط تركيزخضاب الخلية (34±1%)، متوسط تعداد الصفائح الدموية ($269\pm125 \times 10^9/L$) لكل مرضى السرطان اللمفاوي الحاد اقل قيم بالمقارنة مع قيم متوسط المعيار متوسط خضاب الدم (12±1g/dl)، متوسط عداد كريات الدم ($5\pm5.2x10^6/\mu$) ،متوسط تكدس الخلايا(36±3 %)، متوسط تعداد الصفائح الدموية ($353\pm8 x10^9/L$) بفرق ذو دلالة إحصائية (p=0.00)، أما متوسط حجم الخلايا (84±12fl) ،متوسط خضاب الخلية (28±2pg) ،متوسط نسبة الخلايا اللمفية (54±17%)، متوسط نسبة الخلايا المريضة (21±8 %) ومتوسط تعداد كريات الدم البيضاء ($19\pm51x10^3/\mu l$) لدى المرضي اعلى قيم بالمقارنة بالمعيار، متوسط حجم الخلايا (81±3fl)،متوسط خضاب الخلية (27± 1pg) متوسط نسبة الخلاياللمفية (39± 9%) ومتوسط نسبة الخلايا المريضة (0.00%) بفرق ذو دلالة احصائية (p=0.00). خلصت الدراسة ان مرض سرطان الدم اللمفاوي معظمهم من الذكور وأن المرضي لهم فرق من المعيارعند تحليل الدم الكامل لهم . وأن اكثر فئة مصابة مابين الاعمار 10-6 سنه وأن الطراز الظاهري B(75.5%) هو الاكثر شيوعا.

Introduction

Acute lymphoblastic leukaemia (ALL), a group of onco-haematological, quickly developing, clinically, and biologically heterogeneous entities is a cancer of the white blood cells (WBCs) (Jiménez-Morales et al., 2017). It is the most common in children, representing around 73% of all leukaemia cases, 25% of all childhood cancers and approximately less than 1% of adult cancers (Hunger and Mullighan, 2015, Al-absi et al., 2017, Zheng et al., 2017). It has various rates in both childhood and adults. The rate of childhood malignancy and type differ to a large extent all through the world (Pesaresi, 2019). It is increasing crosswise Africa and the Middle East as consciousness increased (Ibrahim and Osman, 2011, Awadelkarim et al., 2012). The reports of the lower leukaemia frequency in sub-Saharan Africa perhaps is due to the breakdown of diagnosis or un-systemic reporting to some extent (Saeed et al., 2014). In Sudan, Leukemia is the second type of the ten top most frequent malignancies in Khartoum, and ALL represented the second kind of leukaemia (Ahmed et al., 2014, Churchman et al., 2016).

Acute leukaemia (AL) is classified in several methods. The French, American, and British (FAB) classification which classified AL into ALL and Acute myeloblastic leukaemia (AML), is an ideal one that depends on the morphology and cytochemical staining of blasts (Ali, 2015).

However, the new classification systems suggested by the World Health Organization (WHO) require the additional evaluation of the leukemic blasts by molecular analysis and flow cytometry. ALL have two universal immunophenotypes; T-cell and B-cell precursors; B-cell represents about 88% of all cases. The two precursor cells present in both children and adults. The category-zation is described by gatherings of original chromosomal alterations, morphol-ogic and immunologic features of the blasts, cytochemical stains and cell types by flow cytometry (Errahhali et al., 2016, Bashasha et al., 2017, Gupta et al., 2019).

The lymphoblasts have an overstated and uncontrolled growth, disordered immune response and defective bone marrow production of normal cells that leads to a deficiency of circulating red cells (anaemia), platelets (thrombocytopenia), and neutron-phils (neutropenia) (Munir and Khan, 2019). The setting in which lymphoblasts is seen in the peripheral blood and bone marrow aspirate can vary significantly. In the majority of cases, the counts and cellularity are high, but in some, there can be pancytopenia, and hypocellularity, which make the recognition of the blasts more critical (O'Malley et al., 2018, Iwafuchi, 2018).

This study aimed to measure the haemat-ological parameters and relate them to demographic data (age, gender) in ALL and control, and to detect the immunophenotypic type (B and T cell). Also, to identify the Philadelphia chromosome [t (9, 22) (q34; q11)] *BCR-ABL1*, in Sudanese patients with ALL.

Subjects and Methods

This was a case-control study that included 140 patients attending Khartoum Oncology Hospital (KOH) in the period from August 2016 to March 2018who were diagnosed as ALL. All diagnoses were made by morphology, Flow cytometry, immunoch-emistry and the Philadelphia chromosome [t (9, 22) (q34; q11)] *BCR-ABL1*using PCR method (QIAamp-RNA Blood Mini Kit) (by QIAGEN, Germany). Blood samples from 140 apparently healthy subjects without a medical history of cancer or other chronic diseases attending Khartoum Teaching Hospital Laboratory Department in the same duration. All patients and controls were of Sudanese ethnicity and matched for age and gender.

Haematological Tests:

Venous blood samples (3- 5 ml) were collected under sterile conditions from each case and control into labelled EDTA vacutainer tubes for complete blood count and blood film. Haematological parameters were studied using an automated particle cell counter (Sysmex KX 21N, Model 250VT2A, and Japan). The phenotypic assessment began with cytochemical studies, and especially with Sudan black B reaction. The majority of immunophenotyping studies were being carried out from blood or marrow aspirates with surface and cytoplasmic markers by flow cytometry (BECKMAN COULTER EPICS XL, System 11 software, USA). In ALL as general, we usedCD34, HLA-Dr, CD45, cCD3 and cCD79a.When cCD97a gave a positive result, was used CD10, CD19, surface and cytoplasmic (Kappa, Lambda, IgM and IgG), TdT and CD22 as B-cell markers. When cCD3 gave a positive result, was used sCD3, CD4, CD8, CD5, CD7, CD1a and CD2as T-cell markers.

Data collection and analysis:

Data were collected using hospital records system in a purposeful data collection form, including demographic data (age, gender). Data were statistically analysed using the Social Package of Statistical Sciences (SPSS, v23).

Results:

The frequency of ALL among the age group (6-10 years) was slightly higher (34.3%) than that registered among younger age group (1-6 years) (28.6%), adolescent (11-15 years) and elderly group (>15 years of age) as shown in table 1. The frequency of B and T immunophenotypes is shown in Figure (1). B and T- immunophenotypes were insignificantly distributed between age groups (p=0.97). The mean of some complete blood count parameters of ALL patients (Hb=10±1 g/dl, RBC=4±1x 10^6/µl, HCT=34±1%, PLT=269±125x10^9/L) was significantly lower (p=0.00) than that in controls (Hb=12±1,g/dl RBC=5±5.2$10^6$/µl, HCT=36±3%, PLT=353±8x10^9/L) and the mean of the other parameters (WBC=19±51x10^3/µl, MCV=84±12fl, MCH=28±2pg, Blasts=8±21%) was significantly higher (p=0.00) than that in controls (WBC=7.6 ±2x10^3/µl, MCV =81±3fl, MCH=27±1pg, Blast=0.00%) as demonstrated in Table (2). The mean age of all subjects was 11 years, with a peak at ~1-10 years old (Figure 2). The number of ALL patients distributed according to gender is shown in (Figure 3). Philadelphia chromosome was positive in 4.3% among children (1- 18 years) and 30.1% in older age (>18 years).

Table (1): Frequency of the Sudanese ALL patients according to gender and age groups.

Characteristic	Frequency	Percent%
Gender		
Male	88	62.9%
Female	52	37.1%
Age group		
1-5 year	40	28.6%
6 - 10 year	48	34.3%
11- 15 year	32	22.9 %
16 -20	5	3.6%
21 -30	8	5.7%
>30	7	5%
Total	140	100%

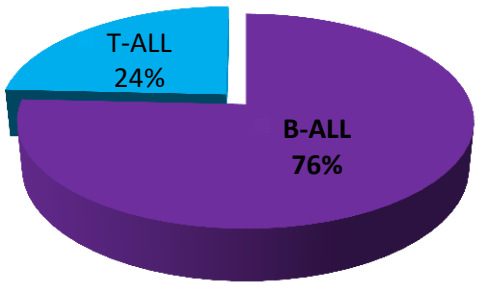

Figure (1): Frequencies of immunophenotypes of ALL among the Sudanese population.

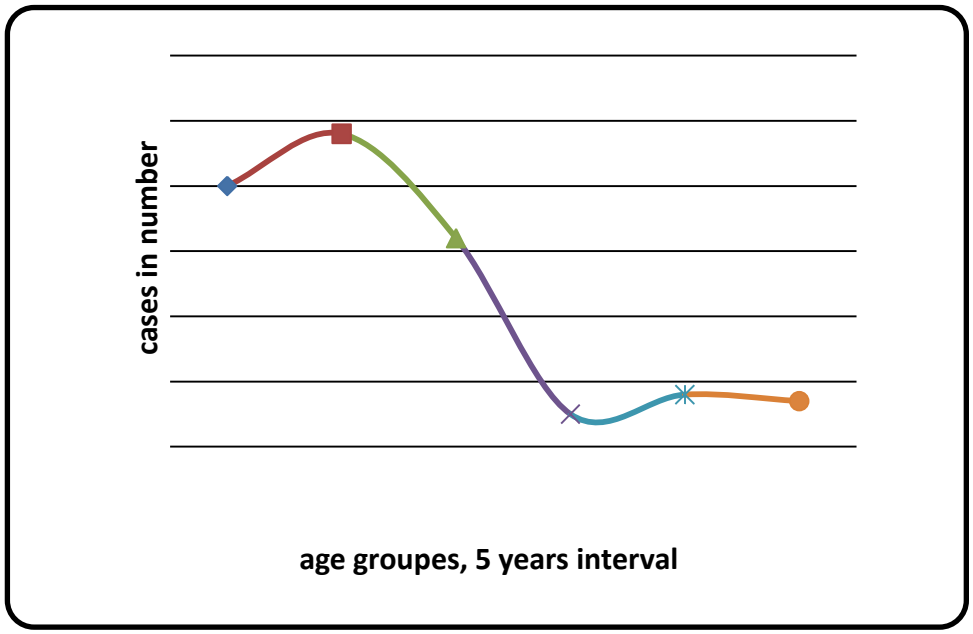

Figure (2): The number of studied ALL patients distributed according to age. Note the peak at ~1-10 years' old.

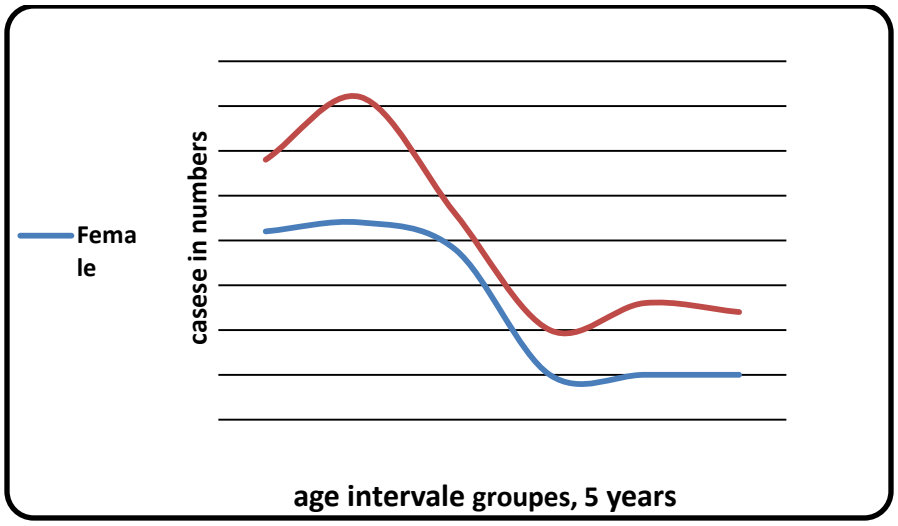

Figure (2): The number of ALL patients distributed according to gender.
Note the female gender was not reported in more than 20 years' age group.

Table (2): Hematological parameters of the studied ALL patients and healthy controls.

Variable	WBC $x10^3/\mu l$	RBC $x10^6/\mu l$	Hb g/dl	HCT %	MCV/ fl	MCH/pg	LYM %	PLTx $10^9/L$	Blast %
Case: Mean ± Sd	19±51	4±1	10±1	34±1	84±12	28±2	54±17	269±125	8±21
Control: Mean ±Sd	8±2	5±5.2	12±1	36±3	81±3	27±1	39±9	353±8	00±0
P .value	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00

Independent *t*-test and mean ±SD.

Discussion

The incidence of malignancy has significantly risen recently in Sudan, and cancer is ranked as the leading cause of the increased mortality in most instances (Ahmed et al., 2014). Childhood cancer, during the early on life, shows demographic and histologic individuality that is different than viewed in older children. Leukaemia was the major malignancy in children less than 15 years of age and adults 15 years and older in Khartoum, and acute lymphocytic leukemia is the common one (Saeed et al., 2014, Saeed et al., 2016, Osman et al., 2019). In this study, the males' frequencies were higher than for females; however, there was a female occurrence among very young children until ~17 years and demonstrated zero rates above those years. This variation was most robust between children 6-10 years of age and younger. The feature at peaks in the 1-10-year age group reproduced more than a 2-fold difference, which was not visible after 20 years of age.

Leukemia incidence usually raises with age (Panagopoulou et al., 2019). The childhood ALL frequency in manycountries, like in UK and USA, have age-specific occurrence patterns that are distinguished by a peak between the ages of 1 and 4 years, followed by declining rates passing during later childhood, adolescence and young adulthood (Cowell et al., 2019, Smith, 2019). The present analysis, however, showed a different profile with a broad peak occurring at 1-10 years, and this result is partially in agreement with Ibrahim et al. Who found 58% of ALL Sudanese patients in the age group 6-16 years (Ibrahim and Osman, 2011).

The morphologic recognition of lymphoblasts in the blood and bone marrow and their phenotypic characterization are of significant importance to the correct diagnosis and classification of ALL. The current study denoted that these abnormal cells are arrested in all the new cases with significantly raised counts and lymphoblasts ($p = 0.00$ of both). However, some cases showed normal cells with features of low white blood cells and low platelet count; mostly because they are under a medication program. Also, variable degrees of anemia with low levels of Hb and HCT were observed in all most all cases although the MCV and MHC appear as normal; this may be due to the folate drug intake by patients. The results of the current study are in keeping with the published literature (Jaime-Pérez et al., 2019, Nakayama et al., 2019).

According to the immunological criteria, ALL is separated into two subtypes; B-ALL (78%), is defined by the presence of either strong CD19 along with one other strongly

expressed B- cell markers and T-ALL (24%), is diagnosed based on the presence of cytoplasmic CD3 in leukemic blasts (Gupta et al., 2019), these agree with our study owing the B- ALL signified as high number than T- ALL without distribution influence within gender or age group among patients (P=0.1).

The Philadelphia chromosome t(9/22) is the outcome from a translocation relating to the breakpoint cluster area of the BCR gene on chromosome 22 and the ABL gene on chromosome 9, PCR analysis exposed rate of 20-30% BCR-ABL+ALL in adult compared with 3% in childhood ALL (Estey et al., 2014). Despite cytogenetic abnormalities are not connected with predictive variables for predicting the result of adult ALL, the translocation can either guide to a p190 BCR-ABL1 fusion protein (ordinary in children) or p210 BCR-ABL1 fusion protein (ordinary in adults)(Horowitz et al., 2018) . The present study showed that the Philadelphia chromosome positivity is slightly increased in Sudanese children and older age (P=0.00); this variation differs from reported incidence (Azevedo et al., 2019, Handgretinger and Döring, 2019).

Conclusion

Acute lymphoblastic leukaemia shows male predominance and differs significantly in haematological parameters than unaffected Sudanese children as in published literature. The adolescents are the more affected age group, and the female's age group has limited representation in more than 20 years. The B-phenotype represents a high-frequency rate. The incidence of Philadelphia chromosome is slightly higher than reported in the literature.

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Ethics Consideration:

Ethical approval for this study was obtained from the Medical Ethics Committee of the Ministry of Health – Khartoum State (The acceptation number: 61–1522). Informed consent has been waived since only the laboratory remaining blood samples were used.

Competing interests: Authors declare they have no competing interests.

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