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Keywords: *sudanese. egyptian childern, acute lymphoblastic leukemia , flowcytometry.*

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Immunophenotyping of Acute Lymphoblastic Leukemia in Sudanese Children's Versus Egyptian Children's using Flowcyometery

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Abstract- Background: Acute lymphoblastic leukemia (ALL) develops rapidly, creating immature white blood cells (WBCs) called lymphoblasts. This type of hematological malignancy is cancer involving blood and bone marrow (BM). It can affect both B and T cell lineage. Here we studied T & B markers in 100 Sudanese ALL patients attending the Radiation & Isotopes Center Khartoum versus 180 Egyptian ALL patients attending the Alksr Aani, oncology center, Cairo between October 2009 and August 2014.

The study aimed to detect the frequency of B and T lineages subclass in ALL among the Sudanese versus Egyptian population in correlation with their clinical symptoms, hematological parameters, gender and, age.

Materials and methods: A questionnaire was use to collect geographical information and, clinical data. Initially, full blood count (FBC), peripheral blood morphology and BM examination was done to diagnose patients. FBC was done by a haematolgy analyzer and Leishman stain was use for the cell morphology. Immunophenotype CD markers, TdT, CD3cyt, CD3 surf, CD5, CD7,CD2 , CD10, CD19, CD20, Ig , sIg, and ,CD2 antigens were looked for to determine the B and T subclasses of ALL using flow cytometry. Mononuclear cells were prepare for flow cytometry and labeled with fluorescence conjugated antibodies. Analyzing gated populations with EPICS XL using three-color protocols, fluorescence intensity and peak width were calculate for each antigen. TdT, CD10 and, CD19 were positive in all subclasses of B lineage ALL.(((sIg and Ig-cyto were detected in B ALL and Pre B ALL were detected the former was sIg .In contrast, sIg and Ig-cyto markers were not detected in cases with Pro B ALL. Panel T CDs were detect in thymocyte and post thymocyte ALL, as the former was positive CD3 (cyto) and the latter was positive CD3 (suf). TdT, CD2 and CD5 immunophenotypes were positive in all subclasses of T-ALL))). Pro B ALL was diagnosed in 24% (n=43) and 23% (n=23) of cases, 51% (n=91) and 29 % (n=29) were Pre B ALL, 9% (n=16) and 29% (n=29) BALL, 4% (n=8) and 11% (n=11) thymocyte ALL and, post thymocyte ALL was 12% (n=22) and 9 % (n=9) among Egyptian and, Sudanese patients respectively.

Result: Patients were classified into three categories based on their ages, (1-4 Yrs), (5-8 Yrs) and (9-12 Yrs). The frequency of ALL in the first group was significantly higher in Egyptian than in Sudanese children ($p<0.05$) ,while in the last group (9-12 yrs) , it was significantly higher in Sudanese children ($p<0.05$). Pre-B ALL was significantly higher in Egyptian than in Sudanese ($p<0.05$), particularly in those less than 8Yrs, whereas BALL was significantly higher in Sudanese in comparison with Egyptian ($p<0.05$). With regards to prevalence of T lineage ALL (thymocyte and post thymocyte) and, cases of B lineage ALL cases (Pro, Pre and B ALL), there was no significant variation ($p>0.05$) between the two ethnic groups Sudanese and Egyptian. Their significance is represented in parenthesis. You mentioned a significant difference. Regarding hematological parameters, the means of Hb concentration, TWBC, platelets and, lymphoblasts for patients with ALL were 9 and 8 g/dl, 37X l03/cm and 32Xl03, 64X103/cm and 65X103 /cm and, 75%and 72% in the Sudanese and, Egyptian respectively. The presence or absence of lymph node were stratified this way, pro B ALL, pre B ALL, B ALL, thymocyte ALL and post thymocyte. Their presence were (12%) , (13%), (14%), (5%) and (8%) In the Sudanese patients while in the Egyptians it was (19.5%), (44%), (7%),(9.5%) and (4.5%). Flow cytometry has a distinctive role in the diagnosis and differentiation of ALL. Diverse flow cytometric parameter use helps minimize marker numbers leading to reduced cost without reduced accuracy.

Conclusion: Age ranging from one year to 12 years with a mean of 6.5 years. The male to female ratio was 1:37 High age group in Egyptian ethnic group was 1—4 Yrs while in the Sudanese ethnic group, the higher age group was 9-12 Yrs. In this study, B lineage origin is the most common type than T lineage origin in two ethnic groups: the T lineage had a better prognosis than B lineage. In this study, also thymocyte ALL with cytoplasmic CD3 in the pediatric group below two years showed with the high total leucocytic count. Flow cytometry has a distinctive role in the diagnosis and differentiation of ALL.using of certain flow cytometric parameters can helps in minimization of cost without reduced accuracy. There is significant variations in ALL subclassification between Sudanese and Egyptian Patients that may be due to genetic background.

Keywords: sudanese. egyptian childern, acute lymphoblastic leukemia , flowcytometry.

I. Introduction

Contemporary research on childhood acute lymphoblastic leukemia (ALL) has focused on the identification of biological and clinical prognostic markers to generate better risk- adapted treatment strategies [(1)] The identification of several cluster

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differentiation markers and early diagnosis allowed the definition of patient subsets with distinct prognostic features [1] Nevertheless, treatment itself remains one of the strongest prognostic factors, as has been shown in several well-designed large clinical trials [2]. Cytometry has evolved from a promising new technology to an indispensable tool in the diagnosis of hematologic malignancies. Many new antibodies, improved gating strategies, and routine use of multiparameter techniques have dramatically improved the diagnostic utility of flow cytometry. This review will focus on the use of flow cytometry in the routine clinicopathologic approach to the diagnosing of leukemias and lymphomas, emphasizing the relevant literature of the past ten years. Some of the recent advances in flow cytometric monitoring of disease and treatment are shown in the last section. We will review the use of flow cytometry in the diagnosis of major disorders highlighting the prognostically important subgroups defined either morphologically or genetically. The discussion will focus not only on the use of flow cytometry in the differential diagnosis of a particular disorder, but also correlate immunophenotypic, with clinical features, HB, TLC. Platelets and accumulation of blast cells. In the delineation of biologically important subgroups. We intent that this review supports a combined modality approach to the daily practice of hematology-oncology and hematopathology. A working knowledge of the basics of flow cytometry is assumed; thus, technical aspects of instrumentation, normal distribution of surface antigens, and methodologies. leukemia is a group of neoplastic diseases of blood- forming cells of the bone marrow, which result in the proliferation and accumulation of immature and generally defective blood cells in both the blood-stream and the bone marrow [3]. This may result in anemia, thrombocytopenia, and granulocytopenia and, infiltration of other sites such ,as lymph nodes, kidney, spleen, testes, and the central nervous system (CNS). The cells involved are usually leukocytes, but several different forms of the disease may be manifested; according to which leukocyte cell line is involve, the leukemia are universally fatal if untreated, generally due to complications resulting from the leukemic infiltration of the bone marrow and replacement of normal hematopoietic precursor cells. These fatal complications are usually hemorrhage and infection [3]. Leukemia is the most common childhood cancer, accounting for one- third of malignancies in children under 15 years of age in Europe and North America. The annual incidence in the United Kingdom is 3 0-40 cases per million children. About 80% are acute lymphoblastic leukemia (ALL) and 18% acute myeloid leukemia (AML) [4] . Acute lymphoblastic leukemia, with a sex ratio of 1 .2 males to females, shows a peak incidence in childhood between the ages of 3 and 5years and is due to cases of early B cell ALL. T-ALL, with a male predominance, is more common in older children ('Stiller and Draper 1998). Both incidence and mortality are slightly higher in males [5]. A peak of age occurs between 2-4 years (Margolin and Popolack 1997). in Egypt, acute leukemia is the most common pediatric malignancy accounting for about 40% of childhood cancer, with ALL counting for 70% of the cases [5]. the peak incidence is between 3-7 years [6] .

II. Materials and Methods

Study Participants; This study included 180 Egyptian children and 100 Sudanese children newly diagnosed with acute lymphoblastic leukemia, 158 males and 122 females. Their age ranged from one year to 12years.

Sample preparation and methods

For each patient, the following samples were collect: 4ml EDTA blood sample was collected under complete aseptic conditions for CBC and BM aspirate for morphological examination and immunophenotyping on EDTA containers. BM samples were processed within a few hours because CD antibodies monoclonal have short stabilities even if stored in the refrigerator. In most cases, samples were processed within 6 hours of collection.

Methods; Complete blood count Was done by using Sysmex KX-21N, Kope, Japan. *Principle of smear preparation* A small drop of blood is place near the froste end of a clean glass slide. A second slide is used as a spreader. The blood is streaked in a thin-film over the slide. The slide is allow to air-dry and is then stained.[7]. *Staining of thin film* Leishman stain was used in staining of all blood smears and bone morrow in this study., *Flowcytometry,* The EDTA anticoagulated BM sampled were diluted 1.3 with PBS then the cells were stained by adirect immuno-florescent technique by addition of monoclonal antibodies (microwell Test Kit contains) which contains T lineage panel monoclonal antibodies (CD2, CD5, CD3 cyt, CD7 and, CD3surf) and B lineage panel monoclonal antibodies (CD10, CD9, CD20, CD19 Ig cyt and, Ig surf.) and Leukocyte marker (TdT). Sample staining be carried out as soon as possible after the nucleated cell suspension has been prepared. Delaying this step will only reduce viability and induce cell clumping, especially if the tubes holding the cell suspensions will be stored in an upright position [7]. *Direct immune fluorescence double staining* Tubes was labeled with the name of patients, type of the specimen, laboratory number and combination of fluorochrome. 100 µl of specimen (whole peripheral blood or bone marrow) was placed in a labeled tube., 2ml of phosphate buffered saline PBS, (PH 7.3) was added containing 0.02% sodium azide 0.02% Bovine albumin., Tube was centrifuged at 2000 rpm for 5 minutes and, the supernatant were removed., The cells was resuspended in 02-0.5 ml of fluid sheath solution (e.g . isotonic). Then the tube was readied on a flow cytometer instrument[8]. *Detection of surface Immunoglobulin,*

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Surface 1g heavy and light chain can be detected using Double or triple immune staining the object in to demonstrate clonality of a B cell population. Double staining was done by Combination of an FTC labeled B cell, marker e.g, CD19 and, PE Labeled anti-light chain. The tube was labele with the name of the patient, type of specimen, laboratory number and MCAP. 100 μL of the specimen (whole peripheral blood or bone marrow) was pipette. 2 ml of lysine solution was added, then incubate for 10 minutes at room temperature. The tube was washed twice in PBS aside from BSA. An appropriate Volume of MC Ab was add according to the manufactures recommendation. The tube was re incubated for 10 minutes at room temperature. 2ml of PBS aside BSA or Henks Solution was added. tube was centrifuged at 2000 rpm and, the supernatant was remove. The cell was re suspended in 0.2.0.5 ml of sheath fluid (isotonic) and inserted on a flow cytometer. *Detection of intracellular antigens,* There are several commercially available kits containing solutions to fix and stabilize cells in to detect cytoplasmic and or nuclear antigens, overall, these reagents have little or no effect on the light scatter pattern. Also, their reliability and consistency for detecting particular nuclear and cytoplasmic antigens may vary. The kits contain two solution. A is a fixing solution. B is a stabilizing agent. The tube was labele with the name of the patient, type of specimen laboratory number and, the MC Ab. 100 μL of the specimen was pipette into the tube. 100μL of Solution A (fixative) was added and incubated at room temperature for 10 minutes. Tube was washed twice in PBS and BSA by centrifuging for 5 minutes at 2000 pm. 100μL of solution B (stabilizing) and the appropriate amount of fluoromecen conjugated Mc Ab were added. Cell was incubated at room temperature for 15 minutes .The cell was washed twice in PBs azide BSA by Centrifuging for 5 minutes at 2000 rpm. The cell was resuspended in 0.2-0.5 ml of sheath fluid solution (isotonic) and the tube was inserted on flow cytometer.

Statistical Analysis: Statistical assessment was carried out with statistical package for social sciences (SPSS) version 17.0 for windows statistical software.

III. Results

Age comparison within the study population

In this study, Sudanese and, Egyptian patients with ALL were classified into three categories based on their ages, (1 to 4) Yrs, (5 to 8) Yrs and, (9 to 12) Yrs. Two hundred eighty study participants with ALL, of whom 36% (n=100) were Sudanese and, 64% (n=180) were Egyptian. The frequency of (1-4 yrs) age group was significantly higher in Egyptian than in Sudanese ($p<0.05$). In contrast, the prevalence of (9-12 yrs) age group was significantly higher in Sudanese compared to Egyptian ($p<0.05$). The Pre-BALL was significantly higher in Egyptian than in Sudanese ($p<0.05$), particularly in those less than 8Yrs, whereas BALL was significantly higher in Sudanese in comparison with Egyptian ($p<0.05$). With regards to the prevalence of T lineage ALL (thymocyte and post thymocyte) and cases of B lineage ALL cases (Pro, Pre and BALL), there was no significant variation ($p>0.05$) between the two ethnic groups. Sudanese and, Egyptian. Their significance is represente in parenthesis . Table (1).

Gender comparison within the study population

In this study the majority of children with ALL were males n=170(61%), 66 (66%) were Sudanese and 104(58%) were Egyptian compared to females no=80(39%), where 34 (34%) were Sudanese and 76 (42%) were Egyptian. The Pre-B ALL cases among Egyptian females (patients) were significantly higher than in Sudanese females (patients) ($p<0.05$), in contraste the prevalence of B-ALL was significantly higher in Sudanese compared to Egyptian ($p<0.01$). Their significance is represented in parenthesis. Table (2).

Subclasses of B and T acute lymphoblastic leukemia and their frequencies within the study population

The frequency of B lineage ALL subclasses for the two ethnic groups was 82% out of all studied samples, which was approximately five times higher than those with T lineage ALL subclasses. Pro-BALL was diagnosed in 24% (n=43) and 23% (n=23) of cases, 51% (n=91) and 29 % (n=29) were Pre B ALL, 9% (n=16) and 29% (n=29) BALL, 4% (n=8) and 11%. Table (3).

Immunological findings in the study population

TdT, CD3cyt, CD3 surf, CD5, CD7,CD2, CD10, CD19, CD20, Ig cyt and, sIg antigens were investigate in the differential diagnosis of B-ALL and T-ALL. TdT, CD10 and, CD19 were positive in all subclasses of B lineage ALL. sIg and Ig-cyto are crucial in identifying B ALL and, Pre B ALL as the former is sIg positive and the latter is positive for Ig-cyto. In contrast, sIg and Ig-cyto-markers were not detect in cases with Pro B ALL. Panel T CDs were important in identifying thymocyte and post thymocyte ALL, as the former is positive for CD3 (cyto) and the latter is positive for CD3 (suf). TdT, CD2 and, CD5 immunophenotypes were positive in all sub-classes of T-ALL. Table (4).

Hematological parameters of study population; Four hematological parameters were estimated; Hb, blast cells, TLC and, Plts. This study shows comparative statistics between B and T lineage ALL patients of Sudanese and Egyptian ethnicities. The results were not within the published normal range among study population, as Hb, PLt were low in both ethnic groups and TLC, blast cells were high among them. The difference in these parameters was not significant in Sudanese versus Egyptian patients p = >0.05. Table (5).

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French American British classification: Patients were classified based on the FAB classification: 27% and 17.2% asL1; 62% and 77% as L2 and 11% and 5% as L3 in Sudanese and ,Egyptian respectively. Of Egyptian patients, L2 was highly significant ($p < 0.05$) compared to other classes, L1, of Sudanese patients, L2 was highly significant ($p < 0.05$) compared to other classes while L3 no significant variation in Sudanese and Egyptian. Table (6).

Lymph node in study population: To detect either presence or absence of lymph node. Stratified this way, pro B ALL, pre B ALL, B ALL, thymocyte ALL and. post thymocyte. Their presence in Sudanese were (12), (13%), (14%) ,13 (13%), (5%) and (8%) while in Egyptian were (19.5%), (44%) ,(7%) ,(9.5%) and (4.5%). table (7).

Bleeding; To detect either presence or absence of lymph node and, to bleed for participants. Its presence was stratified this way, pro B ALL, pre B ALL, B ALL, thymocyte ALL and post thymocyte. table (8).

IV. Discussion

A cross-sectional case-control study was carried out at the Radiation & Isotopes Center, Khartoum and the Alksr Aani, oncology center, Cairo to compare the prevalence of ALL immuophenotypes amongst the Sudanese and, the Egyptian population using flow-cytometry. Blood cell morphology, cytochemistry stains, cytogenetic studies and immunophenotyping are basic methods for ALL diagnosis (1, 2). These techniques have been used extensively over several years in different parts of the world and, a wide distribution of hematological malignancies found for different regions (3-5). The data report here are based on flow-cytometry results from blood samples taken from diagnosed Sudanese and Egyptian ALL patients (figure1). Flow cytometry was used to evaluate the different types of ALL, B and, T lineages in the peripheral venous blood and, the detection of their subclasses, based on the Cell 'Cluster of differentiation (CD) markers (6, 7). Here, TdT, CD3cyt, CD3 surf, CD5, CD7, CD 2, CD10, CD19, CD20, Ig cyt and, sIg antigens were investigated in the differential diagnosis of B-ALL and T-ALL lineages and their immunophenotypes, as discussed in Table (4). CD markers are a helpful method to recognize a specific cell population, however; they might be express on more than one cell type (8, 9). This was also found here among study patients with T lineage, as the CD7+ was detected in 47% and 51% of Sudanese and, Egyptian, respectively (See Figure 3). Thus flow-cytometry methods have been develop for immunophenotyping cells with two or more antibodies simultaneously to diagnose subpopulations of ALL effectively (10, 11). Of these markers, sIg, CD10, CD19, CD20 and Ig *cyto* were used in this study is the differential diagnosis of B lineage ALL immunophenotypes. Similar markers have been used in reported studies in the literature review (7, 12, 13). The immunophenotypes of B lineage ALL can be differentiate by analyzing the results for just five CD markers, sIg, Ig-*cyto,* CD10, CD19 and, TdT. As the TdT is positive in all immunophenotypes of ALL, the current study used the first four CD markers in differentiating subclasses of B lineage ALL as follows: CD10+ and CD19+werefound in all subclasses of B lineage-ALL, but they were not found in all immunophenotypes of T lineage ALL; sIg+ was found only in B-ALL and Ig-*cyto*+ was found only in Pre B ALL. In contrast, sIg+ and Ig-*cyto*+markers were not found in cases with Pro B ALL, as discuss in Table .4. This classification is important for the identification of the outcome of the ALL immunphenotypes (14, 15). The immunological sub classification of B and T lineages ALL is important in diagnosis in correlation with clinical features and molecular cytogenetic for management of patients for instance pro-BALL in children is associated with t(4,11) (16), pre-BALL consider coarse prognosis when is accompanied with t(9;22) & t(1;19) (Philadelphia chromosome) (17) and, BALL with translocation of (8;14) (Burket lymphoma) (18, 19). Regarding panel T lineage CD markers in the current study, CD2+ and CD5+ were found in both thymocyte and post thymocyte ALL sub populations but CD3 (cyto) + was found to be positive only for thymocyte ALL and CD3+ (suf) was positive only for post thymocyte ALL (See Table 4). In contrast, this finding was similar to the study by Yoneda, N. and co-workers concerning the presence of the CD2+ and CD5+ in T lineages ALL subpopulations (7). Of the study samples with T ALL, CD7+ was found in approximately half cases of Sudanese and Egyptian. This marker was found in all cases with bad outcomes for T ALL subpopulations, thymocyte and, post thymocyte (13, 20). T lineage ALL (thymocyte &post thymocyte) also has considered with bad prognosis when is associated with t(11;14) & t(10;14) respectively (19, 21). All cases had TdT at the time of initial diagnoses, but other CDs marker significantly increased during the staging of the diseases, such as Ig and CD7. Immunophenotypes also appear to affect the prognosis of ALL (figure 1). It is hoped this study may act as a pilot study to highlight the need to implement a flow cytometry for ALL and other hematological malignancies in Sudan. In the current study, B lineage ALL subclasses were detected more than T lineage ALL, but the former was a little bit more in Egyptian compared to Sudanese, 84% and 80%, respectively. Controversially, T lineage ALL subclasses were detected higher among Sudanese (20%) than Egyptian ethnicities (16%). (See Figures 2 and 3). This finding is in disagreement with other ethnicities, as in patients with ALL from Brazil and Japan. In Brazil, the B lineage ALL was detected in lower frequency 56.7% and the T lineage ALL in higher frequency 43.3% (22), while

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in Japan, the T-lineage ALL accounted for lower frequency (13%) and B-lineage ALL accounted for higher frequency (87%)(37) compared to the current study groups. The higher percentage of B lineage ALL in this study might explain that the outcome of ALL might worsen in Egyptian and Sudanese; as it was reported to have a significantly poor event; low survival compared with patients with B lineage ALL (20). Accurate immunophenotyping of ALL is essential to evaluate the value of treatment in early diagnosis and to individualize treatment protocols, as described in the literature review (22), the frequency of ALL subclasses constitutes the theme of this study, using CDs markers (See Table 4). Pro-B-cell ALL in all age groups is associated with an unfavorable prognosis Patients with pro-B cell phenotype had a more favorable prognosis compared to those patients with pre-B cell phenotype, based on their clinical symptoms (36). Another study reported that there was a significant correlation between immunophenotying at diagnosis and higher complete remission rate and longer survival (24). Of Egyptian patients with B- lineage ALL, the commonest immunophenotype was pre-BALL compared to other B-lineage ALL. In contrast, out of Sudanese patients with B- lineage ALL, the prevalence of B- lineage ALL immunophenotypes was approximately similar with some varying degrees (See Table 3). Accordingly, most Egyptian cases with B- lineage ALL may have bad prognosis, as most of their cases were pre B ALL as opposite of Sudanese cases with B- lineage ALL. Therefore, from the above-mentioned studies and the current findings, the immunophenotyping at diagnosis may predict the a good outcome. The quantity of CD marker was used to evaluate the outcome and staging of ALL, it found that cases with positive CD10 had a good prognosis (25). In this study, the CD10 was detect positive qualitatively in all cases with B lineage ALL. Hence, further researchers might be useful to detect CD10 quantitatively in these ethnicities. The flow cytometry technique was preferred over alternative techniques in this study because of its accuracy and reliability. It is currently reported as one of the most reliable methods for hematological malignancies; it also has the advantage of diagnosing patients with ALL and other hematological malignancies (15, 24). Several antibodies must be used together to evaluate unique cell markers (35), Therefore eleven CD markers were use in the current study to immunophenotype ALL, B and T populations and subpopulations, as demonstrated in Tables 3 and 4. Although flow-cytometry is currently the best available method for ALL immunophenotypes determination, it is expensive of equipment purchase and maintenance. The study is also concern with the high prevalence of ALL in Sudan, as reported previously (25, 26). The data collected was also intended to instigate the relationship between immunophenotypes of ALL between Sudanese and, Egyptian patients (See Table 3). Interviews and questionnaires were designed to collect demographic: age, sex, ethnicity (tribe), family history and clinical data (See Appendix-1). This information was taken from the patients' parents and, information on clinical symptoms, family history and geographical data. ALL is very prevalent in Sudan and Egypt with, high mortality and morbidity rates (25-28). It is worth searching of ALL in Sudan, as it is a fertile and , virgin area due to a lack of researches and the last international published study was done approximately three decades ago by Ahmed and, co workers (25). Thus, the current research intended to identify the frequency of B and T lineages ALL and their subpopulations in Sudanese children with ALL. Of Sudanese cases here, 17% had T lineage ALL and 81.5% had B lineage ALL (See *Table 3*). In contrast, this finding was similar to the study by A. Redalliand co-workers, concerning the widespread presence of the B and T lineages ALL in Italy, the United States (US), Switzerland, and Costa Rica, where ALL was report with the highest incidence (29). Furthermore, in the current study, the prevalence of ALL subclasses among Sudanese was slightly different from the Egyptian findings, 20% were T ALL and 80% had B ALL (See *Table 3*). The former was marginally higher in Egyptian than in Sudanese, whereas the latter was vice versa. Generally, in both ethnic groups, Sudanese and, Egyptian, the frequency of ALL was nearly similar with some varying degrees compared to the previously reported studies (29, 30). In general, the prevalence of B lineage ALL was higher than T lineage ALL phenotypes (31) (32), as a study found that its frequency was 76.8% pre B and 6% as pro B and 2.3% as T ALL (19) what about the rest ?? and , another study was detected the T ALL only 1.3% in newly diagnosed ALL patients which was slightly lower than the previous study. In the current study, T ALL was 20% in Sudanese and 16% in Egyptian, which was higher compared to the above two studies (12) (15) and a little bit than in a study reported by Pieter Van and coworkers (31). BALL was 80 % in Sudanese and 84% in Egyptian, which was slightly lower than were found in the previous studies (31) (20) (16). This prospective study included all newly diagnosed children with ALL less than 15 years of age registered from October 2009 to August 2014 at Radiation & Isotopes Center Khartoum versus 180 Egyptian ALL patients attending the Alksr Aani, oncology center, Cairo. Besides epidemiological data, the objective of the current study was to look for the age, sex, clinical features and, laboratory findings at presentation and compare it with reported literature. Approximately 75% of ALL cases are in children and its after other nervous system and brain tumors (29). The relationship between ALL and, age was report from the published sources found within this Literature Review (30, 32, 33). As the ALL is the most common leukemia among children and the second most common cancer of childhood after

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other nervous system and brain tumors, the current study was carried out in Sudanese and Egyptian children with ALL (29) .A higher mortality rate was report among children with ALL, who were younger than two Yrs and older than 10 Yrs (34) Another study found that 2-6 Yrs children with ALL survived more than those who were less than 2 Yrs and older than 10 Yrs (Ref). ALL in pediatric is treat based on risk factors, which is defined by laboratory and clinical features, therapeutic approach can be provided for patients who have a lower probability of long-term survival (14, 32), so that this study highlight and interpreted the frequency of ALL subclasses among Sudanese and Egyptian population in correlation with their ages, as discussed in (Table 1) With regards to B lineage, the prevalence of BALL in 9-12 yrs group was significantly higher in Sudanese compared to Egyptian ($p<0.05$), whereas the frequency of Pre B subclass among those who were less than 8Yrs was higher in Egyptian than in Sudanese ($p<0.05$) (See Table 3.2). This finding is similar to a reported study by (Smibert 1996 and co- workers, (34) 2-6 Yrs children with ALL were survival more than those who were less than 2 Yrs and older than 10 Yrs (34). This poor outcome in infants may be related to the common occurrence of other poor prognostic features in this group of patients, such as higher leucocytic counts, higher incidence of hepatosplenomegaly and, immunophenotype (13). Patients under the age of three years were found to have significantly lower intelligence quotients than patients who received the same treatment at an older age and a group of healthy children matched for age, sex and, parental occupation. As the intensity of treatment required for favorable outcome varies substantially among subsets of children with ALL, the participant's ages of the two ethnic groups were compared to identify the prognosis of the ALL in each one.

With regards to the ages of patients with T lineage ALL (thymocyte and post thymocyte), there was no significant variation ($p>0.05$) between the two ethnic groups, Sudanese and Egyptian.(Table 2) summarized gender differences in incidence rates of childhood B- and T precursors ALL. Of B lineage ALL, males had higher susceptibility to having ALL rather than females in both ethnic groups, still, in T lineage ALL, the frequency was nearly the same in males and, females of both ethnic groups without significant variation (p-value > 0.05) [See Table 2]. Ching-Hon Pui and , coworkers were reported that boys had higher susceptibility to having T-cell ALL than girls (20.9% *v* 10.7%) (35). This is in agreement with the current data, as males were more likely to have T-cell ALL than females (66% *v* 34%, $P<.001$), in both subclasses of T-lineage ALL, thymocyte and post-thymocyte. Acute lymphoblastic leukemia (ALL) develops at a rapidly, creating immature white blood cells (WBCs) called lymphocytes. This type of hematological malignancy is cancer involving blood and bone marrow (BM) (36).With regards to laboratory data, the Egyptian population showed severe anemia (Hb < 8.8 g/dl), thrombocytopenia ($68X10^3$/cm) and, ~ %69 of them had blast cells. Interestingly, the B-ALL subclass had a higher number of blast cells than other subclasses of ALL and, the thymocyte subclass had a higher percentage of blast cells rather than the post thymocyte subclass (See Table3.5). This data was in agreement with a study of (14), as they found a high percentage of blast cells in cases with thymocyte & pre B ALL compared with other subclasses of ALL. This may reflect that Egyptian patients with ALL have severe clinical symptoms, as they might have a bleeding tendency because of low platelet and anemia as due to low Hb concentration. Recurrent infection is not unexpected in those patients as they had a high number of blast cells in their blood circulation. In contrast, Hb and platelets were found low among Sudanese cases, < 9 g/dl and $65X10^3$/cm, respectively which were the same as in the Egyptian population without significant variation (p> 0.05). Blast cells were detect slightly higher among the Sudanese compared to the Egyptian, ~ %78 without significant variation (p> 0.05) [See Table 5].The presence of lymphadenopathy is usually found in cases with poor prognosis and, patients who have a diagnostic problem (37). Thus it is important to distinguish between cases of ALL with lymphadenitis from others. Clinical features such as lymph node (LN) enlargement and bleeding were taken from each patient using a questionnaire. The presence of LN enlargement was detect significantly higher (p-value <0.05) in Egyptian 85% (N=153) than in Sudanese 52% (N=52). Of Egyptian cases, its presence in B lineage was found high pre B ALL phenotype 44% followed by pro B ALL 19.5% (N=35) and B ALL 7% (N=14) and its presence in T lineage ALL was detected in all cases with post thymocyte and, its approximately three-quarters of cases with thymocyte. Regarding lymphadenopathy among Sudanese, it was found that its presence and absence were nearly the same, 52% and 48%, respectively. There was no significance variation in the presence of lymph node enlargement between the B lineage ALL phenotypes in Sudanese. Interestingly, patients with post thymocyte are possible to have lymphnode enlargement, as found that all Egyptian with post thymocyte had lymphadenopathy and Sudanese patient with post thymocyte had lymphadenopathy except one case (See Table3.6). Therefore, patients with thymocyte ALL phenotype might have a better outcomes in comparison with post thymocyte ALL phenotype. The presence of bleeding was nearly equal in ethnic groups, 53% in Sudanese and 54% in Egyptian. Regarding to bleeding in the study, population, the high frequency was found mostly in the early phenotypes of B lineage ALL in Egyptian and, vice versa in Sudanese patients. Of T lineage ALL, the presence of bleeding was common among Sudanese in both phenotype equally,

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thymocyte and post thymocyte, but its presence in Egyptian was found mostly in thymocyte (See Table 7). In this study Sudanese and Egyptian patients with ALL were classified into three categories based on their ages, (1-4 Yrs), (5-8 Yrs) and the (9-12 Yrs). This Table describes two hundred eighty study participants, with ALL, of whom 36% (n=100) were Sudanese and 64% (n=180) were Egyptian. The frequency of (1-4 yrs) age group was significantly higher in the Egyptian than in the Sudanese ($p<0.05$). In contrast, the prevalence of (9-12 yrs) age group was significantly higher in Sudanese compared to Egyptian ($p<0.05$). The Pre-BALL was significantly higher in Egyptian than in Sudanese ($p<0.05$), particularly in those less than 8Yrs, whereas BALL was significantly higher in Sudanese in comparison with Egyptian ($p<0.05$). With regards to the prevalence of T lineage ALL (thymocyte and post thymocyte) and cases of B lineage ALL cases (Pro, Pre and, BALL), there was no significant variation ($p>0.05$) between the two ethnic groups Sudanese and Egyptian. Their significance is represent in parenthesis.

V. Conclusions

This study concluded that:- Age ranging from one year to 12 years with a mean of 6.5 years. The male to female ratio was 1:37 High age group in Egyptian ethnic group was 1—4 Yrs while in the Sudanese ethnic group the , higher age group was 9-12 Yrs. In this study, B lineage origin is most common type than T lineage origin in two ethnic groups; In the T lineage had a better prognosis than B lineage. In this study, also thymocyte ALL with cytoplasmic CD3 in the pediatric group below two years showed with a high total leucocytic count. Flow cytometry has a distinctive role in the diagnosis and differentiation of ALL. using of certain flow cytometric parameters can helps in minimization of cost without reduced accuracy. There is significant variations in ALL sub classification between Sudanese and Egyptian Patients that may be due to genetic background.

List of abbreviations

AL	Acute leukemia
ALL	Acute lymphoblastic leukemia
ANLL	Acute nonlymphocytic leukemia
ASIp75	Alternative splice I
ASNS	Asparagine synthetase gene
ATRA	All-trans-retinoic acid
BAK	Proapoptotic Bcl-2 family member
BAL	Biphenotypic acute leukemia
BASP1	Brain acid-soluble protein 1
BFM	Berlin-Frankfurt-Munster
BMT	Bone marrow transplantation
CALLA	CD 10 common ALL antigen
CASP8AP2	Caspase 8—associated protein 2
CBF	Core binding factor
CBP	Creb binding protein
CCR5	Chemokine receptor 5
cDNA	Complementary deoxyribonucleic acid
CG	Control gene
CLL	Chronic Lymphocytic Leukemia
CML	Chronic myeloid leukemia
CN	Copy number
CNS	Central nervous system
COG	Children's Oncology Group
CR	Complete remission
Ct	Cycle threshold
CT	Cytotoxic T lymphocyte
Cyt 1gM	Cytoplasmic immunoglobulin M
DFS	Chronic Leukemia
DIC	Disease free survival
DLBCL	Diffuse large B-cell lymphoma

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dNTPs	Deoxyribonucleotide triphosphates
EAC	Europe Against cancer
EBV	Epstain-Barr virus
EGFR	Epidermal growth factor receptor
EGIL	European group for the immunological classification leukemia (EGIL)
FAB	French-American-British classification
FACS	florescence-activated cell sorter
FIST/HIPK3	Fas-interacting serine! threonine kinase
GSTM1	Glutathione stransferase
GVHD	Graft-versus host disease
GVL	Graft-versus-leukemia
HES	Hypereosinophilic syndrome
HR	Hematological relapse
HRG	High risk group
HSCT	Hematopoietic stem cell transplantation
IIUCE9	Human ubiguitin conjugating enzyme 9
I-BFM-SG	International BFM Study Group
IgITCR)	gene Immunoglobulin andJor T cell receptor
IGF-I rec	Insulin-like growth factor I receptor
IGFII	Insulin-like growth factor II
IL-i 5	Interleukin- 15
IPSS	International prognostic scoring system
ITD	Internal tandem duplications
KD	Killo Dalton
KTS	Lysine-threonine-serine
LDH	Lactate dehydrogenase levels
MALT	Mucosa-associated lymphoid tissue
M-CSF	Macrophage colony-stimulating factor
MDS	Myelodysplastic syndrome
MFC	Multiparameter flow cytometry
MHC	Major histocompatability
MIC	Morphology, immunophenotyping and cytogenetics
MLL	Mixed lineage leukemia gene
MPO	Myeloperoxidase
MRD	Minimal residual disease
DLI	Donor leukocyte infusion
MRDv	MRD value
MRG	Medium risk group
MTHFR	Methylene tetra hydrofolate reductase
MTRR	Methionine synthase reductase
NCN	Normalized copy number
NK	Natural killer
NOS	Non otherwise specified
NSE	Non specific esterase stains
PAS	Periodic acid-schiff
PAX-2	Paired-box gene
PB	Peripheral blood
PBMNC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PDGF-a	Platelet-derived growth factor (x-chain
PG	Profile gene
PNH	Paroxysmal nocturnal haemoglobinemia
PTD	Partial tandem duplications
PTLD	Post-transplant lymphoprol iferat 1 ye disorders
RA	Refractory anemia

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RAR-o	Retinoic acid receptor-ct
Reo	Reactive eosinophilia
RISC	RNA-induced silencing complex
RNA1	RNA interference
ROC-cu rye	Receiver operator characteristic curve
RRM	RNA recognition motif
RT	Reverse transcription
RTo rRQ-PCR	Reverse transcriptase- quantitative- polyrnerase chain reaction
SB	Sudan black
SCT	Stem cell transplantation
SE	Specific Esterase
SENSV	Sensitivity
SF-I	Steroidogenic factor 1
s1RNA	RNA-small interfering sequence.
SREBPs	Sterol-responsive element-binding proteins
SRG	Standard risk group
SRY	Sex-determining region Y
Taq	Thermus aquaticus
TCR	T-cell receptor
TdT	Terminal deoxynucleotidyl transferase
TGF-	Transforming growth factor-3
TNF	Tumor necrosis factor
TYMS	Thymidylate synthetase
WHO	World Health Organization
WT	Wild type
WT1	Wilms' tumor gene
WTIP	WT 1-interacting protein
ZF	Zinc finger
p-ME	3-Mercaptoethano1

Declarations

Ethical approval and consent to participant:

Approval of This study was obtained from the hematology department of medical laboratory science (MLS), Omdurman Islamic University and, the ministry of health issued by the local ethical committee Khartoum State, Sudan. Written consent was taken from each member of the study.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated during and analyzed in this study are not publicly available due to Bahri hospital centers ethical policy to protect participant confidentiality.

Competing interest

The authors declare that they have no competing interests.

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Authors contributions

AA and KO and AH contributed in literature search and manuscript writing. AA had the main idea of the study and contributed in manuscript writing, KO contributed to clinic work, AH contributed in statistical analysis. KO and AA supervised the study and critically reviewed the manuscript. All authors read and approved the final draft of the manuscript.

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Figure Legends

Figure 1: Shows T lineage acute lymphoblastic leukaemia

Figure 2: Shows B lineage acute lymphoblastic leukaemia

Figure 3: Result of CD7 in study population

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