ORIGINAL ARTICLE

Comparison of PAX5 and CD79a expression in childhood Precursor B Lymphoblastic Leukemia

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ABSTRACT

Aim: To determine the frequency and evaluate the expression of CD79a and PAX5 in precursor B lymphoblastic leukemia.
Study design: Cross sectional study
Setting of study and duration: Lahore General Hospital and Chughtai's Lahore lab. and the study duration was one year i.e., from 11st July 2015 to 31st June 2016.
Methods: A total of 56 cases were included in the studyafter fulfilling the inclusion criteria. Suitable cases were identified by as per our selection criteria. Paraffin embedded trephine blocks were provided for immunohistochemistry by the Haematology Department of Lahore general hospital, and Chugtai's Lahore lab. Immunohistochemistry was done for PAX5 and CD79a and results were noted. Data entry and analysis was done by using SPSS 20. Quantitative variables were presented by using mean±SD. Qualitative variables were presented by using Frequency table and percentages.
Results: Median age was 6. Mean age at presentation was 6.78 year. Out of 56 cases 31(55.36%) were males and 25(44.64%) were female.Mean age of male patients was 6.51±3.02 ranging from 2 and 13 years. Mean age of female patients was 7.12±3.64 years ranging from 2 and 14 years. A total of 56 cases were studied. Out of these 49(87.5%) were found positive for CD79a. Twelve point five percent (n=7) showed negative results. The expression of CD79a was cytoplasmic. Sensitivity of CD79a is calculated to be 87.5%. While positive predictive value and diagnostic accuracy is 100% and 87.5% respectively.
Conclusion: It is concluded that PAX5 may prove to be a better diagnostic marker in the evaluation of B cell acute lymphoblastic leukemia cases. Therefore it should be included in antibody panel along with routinely used pan B cell markers such as CD19, CD20 and CD79a. PAX5 showed positivity even in those cases which were negative for CD79a and it gave better sensitivity than CD79a. Therefore, the addition of PAX5 in the antibody panel along with other pan B cell markers can lead to more accurate diagnosis.
Keywords: PAX5 ,CD79a, B lymphoblastic leukemia

INTRODUCTION

Leukemias are characterized by the accumulation of malignant white cells, megakaryoblasts and erythroblasts in the bone marrow and blood. Leukemia is mainly classified into acute and chronic leukemia. Acute leukemia is defined as the presence of more than 20% of blast cells in the blood or bone marrow at clinical presentation[1]. Acute leukemia result from clonal proliferation of immature haemopoietic cells[2]. Two major subtypes of acute leukemia are acute myeloblastic leukemia and acute lymphoblastic leukemia[3]. Acute lymphoblastic leukemia is further classified as having B or T cell. The most frequent lymphoid malignancy in children is acute lymphoblastic leukemia[4]. Precursor B cell acute lymphoblastic leukemia consist of immature B lymphoblasts. B cell acute lymphoblastic leukemia cells express B cell antigens[5]. In majority of cases leukemic B cells express CD19, cytoplasmic CD79a, cytoplasmic CD22, and PAX5. Expression of CD20 as well as that of immaturity markers such as TdT and CD34 is also variable according to the stage of maturation[6].

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Until recent past the diagnosis of acute leukemia was based on the morphology and cytochemistry[7]. Blast morphology and cytochemical stains are not sufficient to specify the lineage of any haemopoietic or lymphoid malignancy. Subtypes of each lineage are defined on the basis of clinical and morphological features in association with immunophenotyping by immunohistochemistry and/or flowcytometry and an emphasis towards classification by molecular genetics[8].

Immunohistochemistry of bone marrow biopsy in paraffin embedded tissues represents a strong diagnostic tool. The results of immunohistochemistry can be correlated with the results of flow cytometry and genetic analysis and above all the clinical findings[9]. Terminal deoxynucleotidyl transferase (TdT) and myeloperoxidase (MPO) which are nuclear and cytoplasmic antigen respectively can be readily detected by immunohistochemistry, which cannot be identified by flow cytometry without prior membrane permeabilization[10]. Immunological markers are valuable for leukemia diagnosis and classification, especially when blasts are morphologically undifferentiated or cytochemical stains are intermediate[11]. The aim of immunohistochemistry is to confirm the leukemia, identify the lineage and establish diagnosis. For that a well planned initial panel containing

few antibodies followed by a more specific second set is more useful. CD45 (leucocyte common antigen) is weakly expressed in B cell ALL. To confirm the immature blast stage TdT and CD34 both can be performed. CD20 is widely used as a screening marker for B lineage[12]. If CD20 is negative other B cell markers such as CD79a, CD22 or PAX5 can be used[13]. TdT is the most sensitive marker by IHC. It gives 99% positivity in cases of T ALL and 95% of B ALL. The most commonly used marker for mature B cell isCD20[13]. CD79a may be used as a first line B cell marker for the diagnosis[14]. PAX5 (B cell specific activator protein) is a member of highly conserved paired box domain family of transcription factor. PAX5 expression is then maintained throughout the life of B cell. Its expression down regulates during plasma cell differentiation[15]. PAX5 expression has an additional utility in the diagnosis of B cell malignancies that lack expression of other common pan B cell markers such as CD20 and CD79a[16] (Dong et al., 2004). PAX5 gives better sensitivity for the determination of B lineage as compared to CD79a , CD22 and CD20. The aim of study was to evaluate the expression of PAX5 and CD79a in precursor B lymphoblastic leukemia, and to see which marker would show better sensitivity and diagnostic value.

MATERIAL AND METHODS

A cross sectional study was conducted at Lahore General Hospital and Chughtai's Lahore lab. A total of 56 cases were included in the studyafter fulfilling the inclusion criteria.Suitable cases were identified by as per our selection criteria. Paraffin embedded trephine blocks were provided for immunohistochemistry by the Haematology department of Lahore general hospital, and Chugtai's Lahore lab. Immunohistochemistry was done for PAX5 and CD79a and results were noted. Data entry and analysis was done by using SPSS 20. Quantitative variables were presented by using mean±SD. Qualitative variables were presented by using Frequency table and percentages. Sensitivity, positive predictive value and diagnostic accuracy forPAX5 and CD79a was calculated.

RESULTS

The present study was conducted in hematology laboratory of pathology, Post Graduate Medical Institute, Lahore and Chughtai's lab to determine the expression of PAX5 and CD79a in childhood precursor B lymphoblastic leukemia. Duration of study was one year (1st July 2015 to 31st June 2016). Fifty six cases of precursor B lymphoblastic leukemia were studied for the expression of PAX5 and CD79a. Cases were selected according to our selection criteria from Chughtais's Lahore lab and Lahore General Hospital, Lahore. Median age was 6. Mean age at presentation was 6.78 year. Out of 56 cases 31(55.36%) were males and 25(44.64%) were females. Mean age of male patients was 6.51±3.02 ranging from 2 and 13 years. Mean age of female patients was 7.12± 3.64 years ranging from 2 and 14 years. A total of 56 cases were studied. Out of these 49(87.5%) were found positive for CD79a. Twelve point five percent (n=7) showed negative results. The expression of CD79a was cytoplasmic. Sensitivity of CD79a is calculated to be 87.5%. While positive predictive value and diagnostic accuracy is 100% and 87.5% respectively. On the other hand PAX5 was found 100% positive. Nuclear staining with PAX5 was strong and homogenous in majority of cases. Forty nine out of 56 cases co expressed expression of PAX5 and CD79a while 7 out of 56 cases were found negative for CD79a. Those negative cases were positive for PAX5.

Table 1: Age

Mean	6.78
Median	6
Std. Deviation	3.29
Range	2 to 14
Minimum	2.00
Maximum	14.00

Table 2: Gender distribution

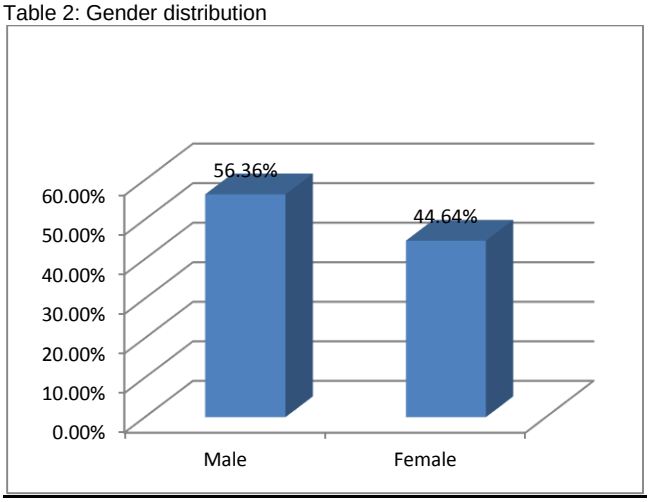

Table 3: Findings of cd79a for diagnosis of precursor B lymphoblastic leukemia

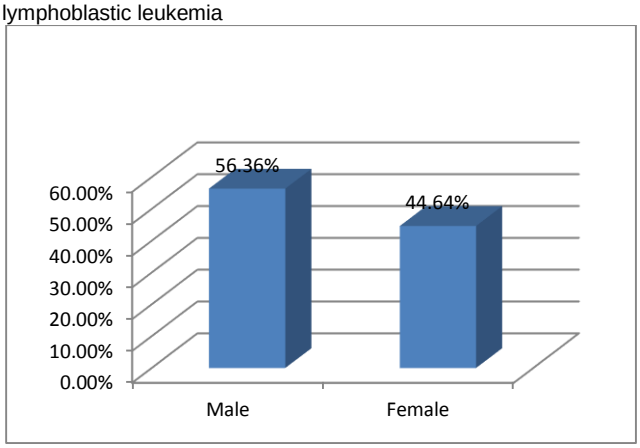

Table 4: Diagnostic comparison of comparison of pax5 and cd79a for diagnosis of precursor b lymphoblastic leukemia

Parameter	Total	Positive	Negative
PAX5	56	56	0
CD79a	56	49	7

DISCUSSION

In past the classification and diagnosis of acute leukemia (AL) was based on morphology of blast and cytochemical stains. Generally leukemias are classified into acute

myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL). Blast morphology and cytochemical stains are not sufficient to specify the lineage of any haemopoietic or lymphoid malignancy. According to WHO for lineage specification antigen expression has to be studied. Subtypes of each lineage are defined on the basis of clinical and morphological features in association with immunophenotyping by immunohistochemistry and/or flowcytometry and an emphasis towards classification by molecular genetics[8]. Immunohistochemistry of bone marrow biopsy in paraffin embedded tissues represents a strong diagnostic tool. The results of immunohistochemistry can be correlated with the results of flow cytometry and genetic analysis and above all the clinical findings[9]. The aim and objective of present study was to determine the frequency, evaluate the expression and usefulness of PAX5 and CD79a in diagnosed cases of B ALL. So that a better marker is added to the antibody panel for the correct diagnosis. It has been observed in previous studies that PAX5 is a better marker for the diagnosis of B ALL. PAX5 shows positivity even in those cases which are negative for other B cell specific markers for example CD19, CD20 and CD79a.Goud et al in 2015 conducted a study on childhood acute lymphoblastic leukemia and found that the median age was 5 years and 7 months at the time of diagnosing leukemia which is approximately similar to the median age in our study which is 6 years. There were 58% males and 42% females which are in accordance to 55.36% males and 44.64% females in our study. PAX5 expression starts in pre pro B cell stage and at pro B cell stage all cells consistently show positivity. PAX5 expression is then preserved throughout the life of B cell. Its expression down regulates during plasma cell differentiation[15]. Numerous previous studies have discussed the probable value of PAX5 in the diagnosis of wide range of B cell neoplasms. Our findings of PAX5 in the diagnosis of B-ALL confirm previous studies that have found this antibody to be diagnostically valued.In the present study PAX5 expression was noted in 56 diagnosed cases of B ALL. The results were positive in 100% of cases. Nasr et al in 2010 did a study on wide range of leukemic cases and they observed that PAX5 was more sensitive marker than CD79a for lineage assignment in B-cell ALL. The results of our study were similar to the results of this study. In this study 34 cases of B ALL were studied it showed 100% positivity for PAX5 and 85% positivity for CD79a. This is almost the same as in our study. In this study the expression of both PAX5 and CD79a was studied and their expression was evaluated. PAX5 was found to be a better marker with 100% positive results for the diagnosis of B ALL. The results of this study were similar to our study which also showed 100% positive results for PAX5 and 87.5% positivity for CD79a.

CONCLUSION

It is concluded that PAX5 may prove to be a better diagnostic marker in the evaluation of B cell acute lymphoblastic leukemia cases. Therefore it should be included in antibody panel along with routinely used pan B cell markers such as CD19, CD20 and CD79a. PAX5 showed positivity even in those cases which were negative for CD79a and it gave better sensitivity than CD79a. Therefore, the addition of PAX5 in the antibody panel along with other pan B cell markers can lead to more accurate diagnosis.

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