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Monoclonal pattern of T-Lymphocytes and B-Lymphocytes receptors gene rearrangement in Acute Lymphocytic Leukaemia in Basrah

Nibras S. Al-Ammar[1], Hermann Kreyenberg[2], Mayada T. Abdulrahman[1]

[1] Microbiology Department, College of Medicine, University of Basrah, Basrah, Iraq
[2] MRD-Chimarism Laboratory, Stem Cell Transplantation Centre, Goethe University Hospital, Frankfurt, Germany

ABSTRACT

Paediatrics clinicians face a problem in evaluation of chemotherapy treatment used for Acute Lymphoblastic Leukaemia (ALL) patients in Iraq. They need an accurate test to evaluate the treatment. This study aimed to detect the clonality pattern of T and B lymphocytes receptors gene rearrangements in ALL patients in Basrah before chemotherapy. Bone morrow aspiration samples from 50 ALL patients used for DNA extraction followed by multiplex PCR by using 42 primers. A total of 147 bands cut from polyacrylamide gel and sent for sequencing in order to detect the pattern of receptors gene rearrangements. 15th ALL patients were found to have rearrangements in the studied genes coding for receptors of T and B lymphocytes; TCR and Ig. Ten patients have a rearrangement of Ig while 5 patients rearranged TCR. Immunoglobulin Heavy Chain (IGH) gene was rearranged in 6 patients. Some patients appeared to have more than one rearrangement in TCR, IGH, IGK, TCRD and TCRG genes, while the rest of the samples have only one rearrangement. The highest clonality appeared in IGH gene in VH1-7/JH, DH1-6/JH and DH7/JH. Studying Ig and TCR gene rearrangements are an important step for testing MRD which consider now a diagnostic method that evaluate chemotherapy protocol treatment and also predict the patient chance of relapse. The current study might be the first step for further work that can help in chemotherapy treatment evaluation in Iraq.

Key words: monoclonal, TCR, IG, gene rearrangement, multiplex PCR, acrylamide gel, ALL

Address for correspondence:
Nibras S. Al-Ammar, Microbiology Department, College of Medicine, University of Basrah, Basrah, Iraq, Email: nibrasalammar@yahoo.com and alammar.nibras@gmail.com

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INTRODUCTION

Leukaemia is characterized by the growth and differentiation of leukocytes abnormally [1, 2]. Acute lymphoblastic leukaemia considered one of serious paediatric malignancies [3]. T-cell Receptors (TCRs) were first described based on their resemblance to immunoglobulin DNA sequences. These are heterodimeric polypeptide chains having constant and variable portions [4]. Acute lymphoblastic leukaemia treatment has made great progress, as have new molecular techniques for monitoring ALL patients throughout their disease genes. It has IGK and TCR genes that rearranged during their development and used to determine clonality and minimum residual illness [5]. Clonality testing substantially aids and facilitates the detection of lymphoid cancers. The T/B cell receptor rearrangement testing based on multiplex PCR is now utilized as a clinical technique to diagnose probable lymphoproliferative illness [6]. Clonal Variable Diversity Joining (VDJ) rearrangement that happens through mutation was used as a marker to indicate the clonality of B and T cells during their maturation [7]. The junctional regions are thought to represent distinct fingerprint-like sequences [8]. Clonal Ig and TCR rearrangements has found practical use of Minimal Residual Disease (MRD) detection, particularly in ALL [9], also have an important role in identifying targets for immunotherapy [10]. The current study aimed to study monoclonal pattern of T and B lymphocytes receptors gene rearrangements in ALL patients in Basrah before chemotherapy. Detection of Ig and TCR clonality patterns is recommended in ALL patients in Basrah. That will make it possible to obtain a reference value in MRD testing.

METHODS

A cohort study carried out from 2020 to 2022 and involved 50 ALL children in Basrah Children Hospital/oncology department, their ages ranged from 1 month-15 years. The inclusion criteria were newly diagnosed children with ALL according to clinical background of each patient includes parameters such as HB, WBC, platelets, blood film and flow cytometry. The exclusion criteria were children with other types of haematological malignancy. Ethical consideration of the approval of the Basrah health authority was obtained previously.

Fresh bone morrow samples (2 ml-3 ml) were collected in a sterile plastic tubes, from newly diagnosed ALL patients which was already available for routine diagnosis in the haematology