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Role of SF3B1 mutation in assessment of acute and chronic lymphatic leukemia

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Introduction:

Leukemia is one of the deadliest diseases having negative impact on people all over the world. Acute Lymphocytic Leukemia (ALL) is a type of WBC cancer, it's a rapidly invasive disease that originates from B- or T-lymphocyte progenitors. Accumulation of lymphoblasts and suppression of normal cells are the main characteristics of the disease course.

Acute lymphoblastic leukemia (ALL) predominantly affects children, with an incidence of 3–4/100,000 in patients under 14 years of age. The five-year survival rate is approximately 90% in children and 65% in adults.

The French–American–British (FAB) categorizes ALL into three subtypes. However, the WHO classification incorporates immunophenotypic and genetic details, offering a more accurate and clinically significant classification, helping to choose therapies and prognostic evaluations according to the distinct characteristics of leukemia. Medical professionals have recently recommended that WHO categorization is better compared to FAB.

Chronic lymphoproliferative diseases (CLPD) are considered as a heterogeneous group of diseases characterized by monoclonal expansion and accumulation of apparently mature lymphocytes, which have a proliferative and/or survival advantage over normal lymphocytes in different organs such as bone marrow, peripheral blood and lymph nodes. This translates into the progressive accumulation of clonal cells and their products, causing peripheral blood and bone marrow lymphocytosis, in addition to lymphadenopathy, splenomegaly or other organomegaly**.**

Recently, mutations in the genes involved in the spliceosome have attracted considerable interest in different neoplasms. Among the spliceosome mutations, those in the *SF3B1* gene are the most frequent and relevant in haematological diseases. Most human genes encode several mRNA isoforms by the alternative splicing process. Because alternatively spliced mRNA isoforms are often associated with cancer, spliceosome mutations have recently sparked significant interest in different neoplasms.

splicing of RNA is a fundamental process in eukaryotes, The spliceosome dynamically assembles on messenger RNA precursors (pre-mRNA), removing introns (regions that do not encode proteins) from pre-mRNA, leaving exons (the remainder of RNA transcription) attached.

It is known that *SF3B1* mutations could play multiple roles in the pathogenesis of tumours by dysregulating several cellular functions and pathways, including heme biosynthesis, mitochondrial metabolism, and the NF-κB pathway.

The messenger RNA (mRNA) spliceosome is composed of a complex of five small nuclear ribonucleoproteins (snRNP) U1, U2, U4, U5, and U6 and other proteins and plays critical roles in regulating mRNA splicing and subsequent protein expression. It is assembled through binding of U1 snRNP to the 5′ splice site of the pre-mRNA, recruitment of U2snRNP to the 3′ splicing site at the intron–exon junction guided by the branch region sequence, and joining of the tri-snRNP U4/U5/U6.

SF3B1, an essential protein in the U2snRNP complex, facilitates U2snRNP binding at the branch point near the 3′ splicing site. SF3B1 mutants were shown to alter U2snRNP function by promoting alternative branch-point usage and induction of cryptic 3′ splice site selection, thereby generating aberrantly spliced mRNA transcripts subject to nonsense-mediated decay and downregulation of target transcripts and protein expression.

The *SF3B1* gene is located on the long arm of chromosome 2 (2q33.1) and encodes for the biggest subunit (155 KDa) of the splicing factor 3b protein complex. The interaction between the *SF3B1* complex, the 12S unit, and the splicing factor 3a complex gives rise to the U2 small nuclear ribonucleoproteins (snRNP), fundamental for spliceosome assembly and mRNA splicing. The SF3B1 protein is located principally in the nucleus, where it forms nuclear speckles; it is necessary to anchor U2 snRNP to the pre-mRNA by sequence—independently binding the intron branch site.

SF3B1 high proportion in hematopoietic malignancies occurs within codon 700 (K700E) located in the HEAT domain repeat sequence. In addition, hotspots such as R625, K666, and H662 also occur in some proportion , which are thought to have similar mutational functions due to their spatial proximity to K700E in HEAT repeats.

The most common *SF3B1* mutation is an A to G transition that results in lysine to glutamic acid substitution at amino acid position 700 (SF3B1-K700E)**.**

Mutated *SF3B1* gives rise to a protein with a neomorphic activity linked to several mechanisms involved in RNA processing. About half of aberrantly spliced mRNAs are characterized by the presence of premature stop codons, resulting in nonsense-mediated RNA decay and downregulation of the protein expression. Although the wild-type allele is still able to be expressed, the amount of protein produced is unable to compensate for the mutant allele. Another possibility is the translation of new proteins that lack the correct biological function and/or that acquire aberrant functions

A study has shown Hotspot K700E and non-K700E *SF3B1^{mut}* MDS show distinct clinical and mutational profiles in MDS, with K700E showing significantly better Overall survival than non-K700E and *SF3B1^{wt}*. Only the absence of the *SF3B1^{mut}* K700E mutation independently predicted worse Overall survival in MDS. Hence, identification of the *SF3B1* mutation type is important for risk stratification.

In the study by Wang and colleges, somatic mutations of *SF3B1* were found in approximately 15% of patients, making this the second most frequently mutated gene in CLL.

In a cohort of 537 unselected CLL cases, *SF3B1* mutations were present in (8.9%) of cases , The residues surrounding Lys700 were the most frequently mutated (48%).

Quesada and colleges in 2012 studied 279 untreated patients with CLL and found *SF3B1* mutations in 27 (10%) **[15]**. the most frequent mutation was K700E, occurring in almost half of the cases, No *SF3B1* mutation was detected in 156 patients with non-Hodgkin lymphoma **[15]**. CLL patients with mutations in *SF3B1* had more aggressive disease and shorter overall survival than patients without these mutations.

Another study provided evidence that the SF3B1 inhibitor H3B-8800 displays therapeutic effects in mouse models of CLL, particularly when used on CLL cells harbouring SF3B1 mutations.

To address the functional role of SF3B1 in T-ALL, SF*3B1 was* silenced in T-ALL cell lines using short hairpin RNAs (shRNAs). The cell proliferation rate was significantly decreased upon *SF3B1* silencing, accompanied by an increase in apoptotic cell death and G_2-M cell cycle arrest. To further assess the role of SF3B1 in disease progression in vivo, *SF3B1* was silenced in luciferase-expressing CUTLL1 cells and transplanted the cells into immunocompromised mice. *SF3B1* silencing led to a significantly reduced tumour burden and prolonged mouse survival.

Subjects and Methods:

1- **Type of the study:** case control study

2- **Study Setting:** Department of Clinical Pathology in Assiut University Hospital.

3- **Study subjects:**

Study will be conducted on peripheral blood samples of 28 acute lymphoblastic leukemia patients, 28 chronic lymphocytic leukemia patients and 28 control.

a. **Inclusion criteria:**

Patients with acute lymphocytic leukemia or chronic lymphocytic leukemia of both genders at any age.

b. Exclusion criteria:

Patients with any other type of malignancies

c. Sample Size Calculation:

Based on determining the main outcome variable, the estimated minimum required sample size is 84 patients (28 patient in each group)

The sample was calculated using G*power software 3.1.9.2., based on the following assumptions:

Main outcome variable is the difference between mean value of Sf3b1 mutation between the groups

1- patients with chronic lymphocytic leukemia

2- patients with acute lymphoblastic leukemia

3- control group free from the disease

Based on clinical experience we expected to find large effect size difference between 3 groups

Main statistical test is one-way Anova to detect the difference between the 3 groups.

Alpha = 0.05

Power = 0.90

Effect size = 0.4

4 –**Study tools (in detail, e.g., lab methods, instruments, steps, chemicals, …):**

- Medical history (age, sex, complaint of the patients)
- Clinical examination emphasizing on hepatomegaly, splenomegaly and lymphadenopathy.
- Imaging: ultrasound.
- Laboratory investigation: Complete blood count (CBC)

  Reticulocyte count and Peripheral blood film.

  Liver and kidney function test

  Prothrombin time, concentration and INR.

  Bone marrow examination.

  Specific laboratory investigation: ARMS PCR for SF3B1 mutation.

Aim(s) of the Research

1- To evaluate the presence of SF3B1-K700E mutation in acute lymphoblastic leukemia and chronic lymphocytic leukemia using PCR technique

ARMS PCR technique will be used to detect the presence of SF3B1-MUTATION in acute lymphoblastic leukemia and chronic lymphocytic leukemia

2-To determine the effect of presence of SF3B1-K700E mutation with other laboratory findings.

determine if the presence of SF3B1-mutation can affect the clinical data as severity of disease or organomegaly and laboratory data as count of white blood cells and platalet and hemoglobin level using automated cbc analyser and bone marrow of the patients (cellularity and count of blast cells) and result of flow cytometry

-Data management and analysis:

Analysis of data by SPSS program. Data will be expressed as mean± SD, number and percentage. Correlation between categorical variables will be performed in 2x2 contingency tables using Fisher's exact test. Comparison between metric variables will be done using Mann-Whitney test and Wilcoxon signed ranks test. Exact p value (2-sided) will be calculated and a value ≤ 0.05 is considered statistically significant.

Ethical Considerations

3.1. Risk – benefit assessment.

Discomfort at the site of puncture. The study is non-interventional in nature. No additional risks are added to our patients

- The results of this study will help to detect the relation between SF3B1 mutation with ALL and chronic lymphoproliferative disorders for better understanding pathogenesis of diseases and possible future modifications in therapeutic strategy

3.2. Confidentiality (dealing with data and data dissemination should be confidential).

- Privacy and confidentiality of all the information will be assured. All data will be confidential and used anonymously for research purposes.

3.3. Statement describing the research procedure to be given to the participants.

The participants will be informed with what we will do to them, be contact with the research team and in any time allowed to withdraw from the research without affecting their treatment plan.

3.4. Informed consent.

Informed consent will be taken.

3.5. Other ethical concerns:

- The research will be conducted only by scientifically qualified and trained personnel.