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A Cross-Sectional Study on Clinicopathological Evaluation of Acute Leukaemias at a Tertiary Care Hospital

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Abstract

Objective: In terms of their clinical traits and physiological traits, Acute Lymphoblastic Leukaemia and Acute Myeloid Leukaemia are two haematological malignancies that are distinct from one another. The objectives of the current study were to identify and classify AL patients and to assess their clinicopathological characteristics using the WHO's 2016 categorization of ALs, revised 4th edition.

Materials and Methodology: 68 persons with an Acute Leukaemia diagnosis participated in this cross-sectional observational study. Through the use of peripheral blood smear analysis, bone marrow aspiration, flow cytometry, and cytogenetic and molecular testing, the diagnosis was made.

Results: Over the course of two years, 68 cases of Acute Leukaemia were discovered, of which 25 cases were Acute Lymphoblastic Leukaemia and 43 cases were Acute Myeloid Leukaemia. According to the WHO's 2016 subclassification of Acute Myeloid Leukaemia, there were 20 cases of Acute Myeloid Leukaemia with Recurrent Genetic Abnormality, 21 cases of Acute Myeloid Leukaemia, Not Otherwise Specified, and 2 cases of Acute Myeloid Leukaemia with Myelodysplasia Related Changes. Twenty PML-RARA positive cases of Acute Myeloid Leukaemia with recurrent genetic abnormality, seven (8;21) RUNX1-RUNX1T1 cases, two NPM1 gene mutant cases, and one biallelic CEBPA case were all examined. Nine out of twenty-one cases of Acute Myeloid Leukaemia with maturation fell into the Not Otherwise Specified grouping. B cell Acute Lymphoblastic Leukaemia was more common than T cell Acute Lymphoblastic Leukaemia in the subcategory of Acute Lymphoblastic Leukaemia. Natural Killer cell Leukaemia cases were more likely to exhibit B cell Acute Lymphoblastic Leukaemia, Not Otherwise Specified group than B cell Acute Lymphoblastic Leukaemia with Recurrent Genetic Abnormality.

Conclusion: Adopting the fourth edition of the WHO 2016 classification fosters uniformity in the reporting of cases of acute leukaemia, which makes it simpler to use targeted therapies and aids in prognosis forecasting. The WHO classification for acute leukaemia is still accurate, useful, and treatment-specific today.

1. Introduction –

An aberrant, unchecked, and extensive proliferation of hematopoietic cells in the body that invade the bone marrow and blood characterizes acute leukaemia, a heterogeneous group of acquired clonal haematological malignancies of uncertain etiology.

Among the several different haematological malignancies, acute myeloid and acute lymphoblastic leukaemia are among the clinically and physiologically heterogeneous phenotypic illnesses.

Acute myeloid leukaemia is more common in adults than in children, although the reverse is also true. Children are affected by acute lymphoblastic leukaemia more frequently than adults. It is crucial to categorize these illnesses so that their biologic behaviours can guide treatment. Standard categorization criteria for acute leukaemia were established in 1976, with the FAB classification being the first of its kind. (Bennett et al., 2014)

Due to issues including subjectivity in morphological subtype assessment resulting in poor concordance and a lack of cytogenetic data, a more refined and comprehensive categorization was required to be incorporated (Muniraj et al., 2015). The WHO classification system changed in 1999 and was updated in 2001, 2008, and 2016. Integrating immunophenotype, cytogenetic, and molecular data was done primarily to increase objectivity and reproducibility. (Karthik et al., 2020, Swerdlow et al., 2016).

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The substantial variety in how acute leukaemia respond to treatment, not merely their biology and clinical history, is what gives them their heterogeneity. Therefore, categorizing these entities in accordance with the updated WHO classification will aid in the prognostication of diseases through the use of targeted medicines.

The current study aims to examine the clinicopathological characteristics of cases of acute leukaemia (acute myeloid leukaemia and acute lymphoid leukaemia) identified at a tertiary care hospital in accordance with the 2016 WHO classification revision.

2. Materials and Methodology

The current cross-sectional observational study was carried out at a Tertiary Medical Centre in Karad by the Department of Pathology. From June 2017 to May 2019, a two-year period, the study was carried out.

Acute leukaemia cases were examined, classified according to the Revised WHO 2016 Classification, and their clinical information, haematological profile, bone marrow aspirate and biopsy results, immunophenotyping, and cytogenetic investigations were noted.

Acute leukaemia cases that met the WHO diagnostic standards for 2016 were included in the study.

Cases of acute leukaemia without advanced ancillary procedures or cytogenetic studies were not included in the study.

In total, 68 patients with acute leukaemia (AML and ALL) participated in this trial. Each participant in the trial gave their signed, informed consent. Patients who were below the age of 18 years had to take informed consent from parents.

A thorough clinical examination, a full hemogram, and a bone marrow aspiration were used to diagnosis each patient. All of the patients who were a part of this study underwent immunophenotyping, molecular analysis, and cytogenetic analysis, and the results were documented.

All cases of acute leukaemia were categorized in accordance with the 2016 WHO classification, 4th revision. The overall examination revealed pallor, fever, generalized weakness, discomfort in the bones, petechiae, ecchymosis, and bleeding gums.

Only a few of the particular systemic examination findings were detected, including lymphadenopathy, splenomegaly, and hepatomegaly. Reports were made about the USG and other radiological findings.

Using the Sysmex XT-1800i, all of the patients received a baseline haematological inquiry that included Hb estimate, TLC, DLC, and platelet count. Smears from the edges were created, dyed, and thoroughly examined for morphology.

Figure 1: Five part fully automated haematological analyser Sysmex-XT 1800-i

Examining peripheral blood smears:

Peripheral venous blood samples were collected in an EDTA-anticoagulated vacutainer, and the peripheral smears were thoroughly examined.

Peripheral smears were produced, coloured, and analysed using Romanowsky Leishman stain. A manual differential count was performed by observing the appearance of mature and immature cells and noting the platelet count.

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In 2 cases of acute myeloid leukaemia where leucopenia was present, buffy coat smears were performed, stained with Leishman stain, and examined for the presence of blast cells. Further investigation was carried out once a preliminary diagnosis was made.

Flowcytometry, molecular, and cytogenetic investigations were all carried out in each case.

- The BD FACS CANTO II/BD FACS DIVA systems were used to perform six-colour flowcytometry.
- Each of these populations underwent cytogenetic investigation using Fluorescence in Situ Hybridization (FISH).
- For molecular analyses of acute leukaemia, polymerase chain reaction (nested PCR/RT-PCR) was employed.
- Records and analyses of the outcomes of molecular, cytogenetic, and flow cytometric testing were made. These results served as the foundation for the WHO's 2016 classification of acute leukaemia.

Bone Marrow Aspiration Study:

Salah's bone marrow aspiration needle was used in every case to collect bone marrow from the posterior superior iliac spine.

The treatment was performed with strict safety measures. Only after administering a 3cc 2% lignocaine local anaesthetic injection prior to performing the surgery. The preparation and air drying of smears. Giemsa stain was used to stain them.

If there were no visible marrow particles in the aspirate, the aspirate was centrifuged, and the smears were made as necessary.

Figure 2: Bone marrow aspiration and biopsy kit

The following factors were carefully checked in bone marrow aspiration smears:

Cellularity, Predominant series, Myeloid: Erythroid ratio, Erythroid series cells, Myeloid series cells, Lymphocytes, Plasma cells, Megakaryocytes, Blast cells, atypical cells, Mitosis, Iron stores and Parasites. To create the myelogram, at least 500 cells have to be counted.

When a dry tap was obtained or the aspirate wasn't enough to make a diagnosis, a bone marrow biopsy was advised. A Jamshidi needle was used to take a bone marrow sample from the posterior superior iliac spine in just six out of a total of 68 instances.

After painting and hanging drapes, 2% lignocaine was injected while adhering to all aseptic precautions. The biopsy needle was cautiously placed into the bone cortex after the stylet was taken out. A biopsy with a maximum diameter of 1 to 2 cm was considered ideal.

The biopsy was taken in 10% formalin fixative, decalcified in 14% EDTA solution, and processed after being treated as normal in paraffin and stained with haematoxylin and eosin.

Statistical Analysis:

The intended proforma was used to record data. The data was entered into a Microsoft Excel spreadsheet, and SPSS version 20 was used for the analysis.

3. Results

68 patients were examined and determined to have

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Acute Leukemia in accordance with the updated 4th edition of the WHO 2016 classification based on total blood count, peripheral smear examination, bone marrow aspirate, immunophenotyping, cytogenetics, and molecular tests.

43 cases of Acute Myeloid Leukemia and 25 cases of Acute Lymphoblastic Leukemia were part of the current study's 68 cases of Acute Leukemia. In the current study, acute myeloid leukemia outnumbered Acute Lymphoblastic Leukemia.

ACUTE LYMPHOBLASTIC LEUKEMIA:

The current investigation comprised a total of 25 instances of Acute Lymphoblastic Leukemia. Males made up the majority (15/25 cases, or 56%) of the age range from which Acute Lymphoblastic Leukemia was diagnosed cases, which was 1 to 14 years. Children were affected by Acute Lymphoblastic Leukemia more frequently than adults. Only four occurrences were found in people between the ages of 31 and 50.

In the current study, B cell acute lymphoblastic leukemia was more prevalent than T cell Acute lymphoblastic Leukemia (6/25 cases; 24%) in cases of Acute Lymphoblastic Leukemia. The results of the current investigation indicated that T-lymphoblastic leukemia, Not Otherwise Specified, was more prevalent in the subclassification of T-cell Acute Lymphoblastic Leukemia (5/6 instances, or 83.3%). Natural Killer Cell Leukemia was detected in just one instance (1/6 patients, or 16.7% of all cases).

The typical age of diagnosis in the current study varied from 10 to 20 years for T-cell Acute Lymphoblastic Leukemia patients, with a mean age of 13.5 years, and from 20 to 30 years for adults, with a mean age of 23.67 years. One patient's Natural Killer Cell Leukemia was found when they were 50 years old. T cell Acute Lymphoblastic Leukemia was more common in men than in women.

Hepatomegaly was the most frequent presenting feature in patients with T cell Acute Lymphoblastic Leukemia, not otherwise specified (n=5), followed by pallor and splenomegaly. In one case, lymphadenopathy was noted.

The majority of B cell Acute Lymphoblastic Leukemia patients in the current study—15/19 cases, or 78.95%—were categorized as B cell Acute Lymphoblastic Leukemia, Not Otherwise Specified.

Fever was seen in 16/19 instances, or 84.21%, of B cell Acute Lymphoblastic Leukemia patients. This was followed by generalized weakness in 15/19 cases, or 78.95%, and bone pain in 11/19 cases, or 57.89%.

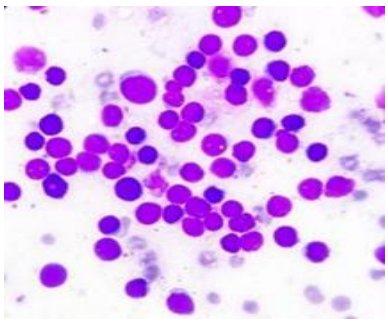

Fig 1. Peripheral blood smear Giemsa stain, 400X

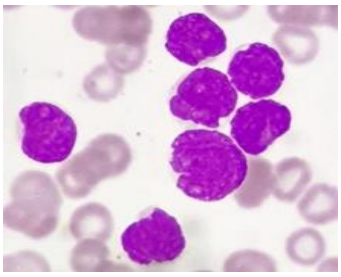

Fig:2 Peripheral blood smear, Leishman stain, 1000X

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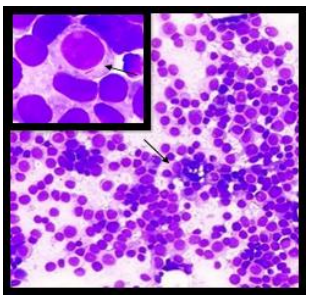

Figure: 28 - PBS shows predominance of large blasts with variable N:C ratio and scant to moderate amount of basophilic cytoplasm. Lack of any maturing granulocytes in this field. Suggestive of AML, NOS (AML without maturation).

4. Discussion

The objectives of the current study were to identify cases with Acute Leukaemia, classify them using the WHO (2016) Classification, and examine their clinicopathological characteristics. Sixty-eight Acute Leukaemia patients were followed up on for two years. In keeping with past studies, Swerdlow et al. (2016) found that more Acute Myeloid Leukaemia cases than Acute Lymphoblastic Leukaemia patients were found. According to study by Dores et al. and others, Acute Myeloid Leukaemia occurred more frequently in adults (aged >30 years), but Acute Lymphoblastic Leukaemia occurred more frequently in children (aged 1 to 14 years). These investigations also showed a connection between the reported symptoms and the outcomes. Fever was the most prevalent symptom in all patients with Leukaemia, similar to Kumar et al., Ghosh et al., and others (72-89%). This was probably brought on by concurrent infections and granulocytopenia. (Greaves, 2016, Azar et al., 2016)

This study also discovered links between bleeding symptoms and generalized weakness. Gingival bleeding was not seen in any of the ALL cases, in contrast to the findings of the studies by Ghosh et al. (23%), Preethi CR (25%), and Sultan et al. (22%), however it was common in AML cases. Ghosh et al. (2003), Camitta et al. (2000), and Greaves et al. (2016) have all cited thrombocytopenia and neutropenia as the causes of this. Pallor was the most prevalent presenting symptom in both ALL and AML, and, like the studies stated above, it was followed by hepatosplenomegaly, which was likely brought on by organ invasion in both cases. (Firkin et al., 2008, Ghosh et al., 2003). The elevated peripheral smear blast cell percentage (68% in ALL and 61% in AML) is also similar to what Kumar et al. and Ghosh et al. observed (85% in ALL and 57.6% in AML, respectively).

Acute Lymphoblastic Leukemia (ALL)

B-cell ALL (B-ALL) was found to be more common than T-cell ALL (T-ALL) in accordance with the prevalence of B-cell ALL (B-ALL) reported by Kumar et al. (63%) (Firkin et al., 2008). The majority of B-ALL blasts were CD10 and CD19 positive. Within 5 days of admission, a patient with posterior reversible encephalopathy syndrome, a normal karyotype, and CD56 aberrancy died from B-ALL that was not otherwise defined (NOS). When the cranial nerve system (CNS) is affected, all patients have a worse prognosis, according to research by Swerdlow et al. and Arber et al. (Campo et al., 2008). This is consistent with what they found. In most B-ALL cases, the genetic anomaly t(12;21)(p13;q22) is present. Blast cells were shown to be positive for CD19, CD10, CD34, and CD13 by TEL-AML1 (ETV6-RUNX1) analysis, but frequently negative for CD20, CD9, and CD66c.

Molecular remission enabled the cure of more than 90% of these patients. Blast cells usually exhibited CD25 at a high frequency, but BCR-ABL1 was more common in adults, according to Campo et al, 2008. Even with imatinib therapy, mortality was discovered three months after diagnosis. Their prognosis is typically the worst of all B-ALL RGA sufferers. Patients with T-ALL had results for CD2 and CD7 that were positive. A 50-year-old male patient with T-ALL NK had an immunophenotypic profile that included blasts that were cytoplasmic myeloperoxidase (cMPO)-negative, CD45 bright+, and significant leucocytosis with 85–90% large-sized lymphoid cells (having a high N:C ratio, clumped chromatin, and cytoplasmic granules with one or two prominent gated nucleoli). A month

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following the diagnosis, a death was reported. T-LL NOS was first diagnosed with an unfavourable prognosis as well. T-ALL NK has an extremely dismal prognosis, according to more study. (Iqbal et al., 2012, Arber et al., 2017).

The current analysis validates the WHO (2016) categorization as a clinically valid approach for identifying the various types of AL, with a key objective of offering focused therapy. Cytogenetics is now one of the most important diagnostic criteria since recurrent genetic abnormalities have provided insight into the molecular mechanisms underlying leukemogenesis. The implementation of immunotherapy, such as the targeted targeting of CD expression, is made possible by the categorization approach's capability to accurately diagnose these disorders, particularly in patients who are not responding to induction therapies. As demonstrated by the use of Gemtuzumab for CD33-positive AML, monoclonal antibody therapy has lately gained significance as a component of therapeutic regimens. Chemotherapy and radiotherapy patients frequently require stem cell transplantation to replenish their hematopoietic stem cell supply in the bone marrow. (Soupir et al., 2007)

This study has a lot of restrictions because it is a single-centre, cross-sectional study with a limited sample size. Multicentric prospective studies with larger sample sizes and longer follow-up times are encouraged to support the findings.

5. Conclusion

Within the limitations of the study, these conclusions were derived. The FAB categorization of Acute Leukaemia has its own set of issues because it was only based on morphology and was subject to subjective assessments. Due to the inclusion of auxiliary studies including immunophenotyping, cytogenetic, and molecular studies along with morphology in the WHO 2016 classification, subjectivity in diagnosis by FAB classification based purely on morphology is abolished. The results of this study show that the groups with unfavourable prognoses have bleak prognoses. The classification of acute Leukaemia patients into groups with favourable and unfavourable prognoses tells us about the course of cases.

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