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SERUM TRACE ELEMENT IMBALANCE AND EXOSOMAL MICRORNA PROFILES IN EARLY RELAPSE ACUTE LEUKEMIA

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Abstract

Background
Acute leukemia relapse remains a major challenge despite improvements in chemotherapy and supportive treatment approaches. Early identification of patients at increased risk of relapse is essential for improving disease monitoring and treatment decisions. Recent evidence suggests that metabolic disturbances and molecular changes, including trace element imbalance and altered exosomal microRNA expression, may contribute to leukemia progression.

Objective
This study aimed to evaluate the association between serum trace element abnormalities and relapse-related exosomal microRNA profiles in acute leukemia patients.

Methods
An analytical case-control study was conducted at a tertiary care hospital in Lahore, Pakistan. Patients with acute leukemia were divided into two groups: early relapse cases and remission controls. Serum samples were collected for assessment of selected trace elements, including zinc, copper, and selenium. Exosomes were isolated from serum, and expression patterns of selected relapse-associated microRNAs were evaluated using molecular analysis techniques. Clinical, biochemical, and molecular findings were compared between both groups. Statistical analysis was performed to determine differences and possible relationships between trace element levels and exosomal microRNA expression.

Results

Early relapse patients demonstrated significant alterations in serum mineral profiles compared with remission controls. Reduced zinc concentration and increased copper levels were observed among relapse cases, resulting in a higher copper-to-zinc ratio. Exosomal microRNA analysis showed increased expression of miR-155, miR-125b, and miR-21, while miR-126 expression was reduced in relapse patients. The altered trace element pattern showed an association with abnormal exosomal microRNA signatures, suggesting a possible link between metabolic imbalance and molecular changes involved in leukemia recurrence.

Conclusion

The findings suggest that serum trace element abnormalities combined with exosomal microRNA alterations may provide valuable information regarding early relapse risk in acute leukemia. An integrated biomarker approach involving biochemical and molecular indicators may improve relapse prediction and support personalized monitoring strategies. Further multicenter studies with larger patient populations are required to validate these findings and establish their clinical application

INTRODUCTION

Serum Trace Element Imbalance and Exosomal microRNA Profiles in Early Relapse Acute Leukemia

Acute leukemia is one of the most aggressive hematological malignancies and is characterized by uncontrolled growth of abnormal immature blood cells in bone marrow and peripheral blood. The disease mainly includes acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL). Despite major improvements in chemotherapy protocols, targeted therapies, and supportive care, relapse remains the main challenge affecting long-term survival of leukemia patients. Many patients achieve complete remission after initial treatment, but a proportion of them develop recurrence because of remaining resistant leukemic cells that survive treatment and later become clinically detectable. Early identification of relapse risk is therefore important for improving treatment strategies and patient outcomes.

The development and progression of acute leukemia involve complex interactions between genetic abnormalities, epigenetic changes, immune regulation, and changes in the bone marrow microenvironment. Traditional monitoring methods such as morphology, flow cytometry, and molecular testing provide valuable information; however, these approaches may not always detect very early biological changes occurring before clinical relapse. Therefore, researchers are exploring new minimally invasive biomarkers that can predict disease behavior at an earlier stage. Among these emerging biomarkers, extracellular vesicle-derived microRNAs and serum trace element alterations have gained significant attention because of their possible involvement in leukemia progression and treatment resistance (Izadirad et al., 2021; Salehi, 2024).

MicroRNAs (miRNAs) are small non-coding RNA molecules that regulate gene expression by controlling messenger RNA translation and degradation. They influence many biological processes including cell proliferation, differentiation, apoptosis, immune response, and drug sensitivity. Abnormal expression of specific miRNAs has been reported in different types of leukemia and is associated with disease development, prognosis, and treatment response. Some miRNAs function as oncogenic molecules by promoting leukemia cell survival, while others act as tumor suppressors and prevent uncontrolled cell growth. Changes in miRNA expression patterns can therefore reflect the molecular condition of leukemia cells and may provide useful information regarding relapse risk (Garzon et al., 2008; Marcucci et al., 2008).

Exosomes are small extracellular vesicles released by almost all types of cells into body fluids including blood. These vesicles contain biological materials such as proteins, lipids, messenger RNA, and microRNAs. Because exosomes protect their RNA contents from degradation, they provide a stable

source of molecular biomarkers. Leukemia cells release exosomes containing specific miRNA patterns that can influence neighboring cells and modify the tumor microenvironment. These exosomal miRNAs may promote leukemia cell survival, immune escape, angiogenesis, and resistance against chemotherapy. Recent studies have shown that exosomal miRNA profiles may help differentiate disease states and identify patients at higher risk of relapse.

In acute myeloid leukemia, several exosomal miRNAs have been associated with disease progression and poor prognosis. For example, altered levels of exosomal miR-125b, miR-155, miR-1246, and other regulatory molecules have been linked with leukemic cell proliferation and resistance mechanisms. Since exosomes circulate in peripheral blood, their analysis provides an opportunity for developing non-invasive relapse prediction methods. Recent evidence suggests that exosomal miRNA signatures may represent a molecular fingerprint of leukemia activity and could complement existing diagnostic and monitoring approaches.

Along with molecular changes, nutritional and biochemical abnormalities may also contribute to leukemia development and progression. Trace elements such as zinc, copper, selenium, iron, and magnesium play important roles in cellular metabolism, antioxidant defense, DNA repair, immune regulation, and hematopoiesis. Alteration in the balance of these elements can affect normal cellular functions and may influence cancer biology. Zinc is essential for DNA stability, immune response, and regulation of transcription factors, whereas selenium contributes to antioxidant enzyme activity and protection against oxidative stress. Copper participates in angiogenesis and cellular signaling, but excessive copper levels may promote oxidative damage and tumor progression.

Several studies have reported abnormal serum concentrations of trace elements in leukemia patients. Increased copper levels and reduced zinc concentrations have been observed in some patients with acute leukemia, resulting in an increased copper-to-zinc ratio. This imbalance may indicate increased oxidative stress and altered metabolic activity associated with malignant transformation. Similarly, changes in selenium concentration may affect antioxidant capacity and influence the ability of leukemic cells to survive under treatment pressure (Kim et al., 2019; Li et al., 2023). Recent studies have also suggested that trace element patterns may have prognostic importance and may be associated with survival outcomes in acute myeloid leukemia patients.

Although trace elements and exosomal miRNAs have individually shown potential as biomarkers, limited information is available regarding their combined relationship in acute leukemia relapse. Leukemia progression is not controlled by a single molecular pathway; instead, it results from interactions between genetic, metabolic, and environmental factors. Trace element imbalance may influence cellular stress pathways, oxidative damage, and gene regulation, while exosomal miRNAs may regulate the expression of genes involved in these processes. Understanding the relationship between these two biological systems may provide new insight into relapse mechanisms.

Pakistan has a considerable burden of hematological malignancies, but advanced molecular investigations for leukemia monitoring remain limited in many healthcare settings. Patients from regions such as Sindh often present with advanced disease due to delayed diagnosis, limited access to specialized testing, and socioeconomic factors. Tertiary care hospitals play an important role in managing leukemia patients and collecting clinical information for research. However, local data regarding exosomal miRNA patterns and trace element changes in relapsed leukemia patients are still insufficient.

The present study was conducted at a tertiary hospital in Lahore, Pakistan, with the aim of identifying correlations between serum trace element abnormalities and relapse-associated exosomal microRNA signatures in acute leukemia patients. This analytical case-control study attempts to compare patients with early relapse leukemia and control groups to determine whether specific biochemical and molecular changes are associated with relapse development.

The identification of reliable biomarkers for early relapse detection may help clinicians recognize high-risk patients before clinical deterioration. A combined assessment of serum trace elements and exosomal miRNA profiles may provide a more comprehensive understanding of leukemia biology and may support future development of personalized monitoring approaches. Therefore, this study contributes important regional evidence and explores a potentially valuable biomarker strategy for improving leukemia management in Pakistan.

MATERIALS AND METHODS

Study Design and Study Setting

This analytical case-control study was conducted to evaluate the association between serum trace element abnormalities and exosomal microRNA expression patterns in patients with acute leukemia who developed early relapse. The study was carried out at a tertiary care hospital located in Lahore, Sindh, Pakistan. The hospital provides specialized hematology services and manages a large number of leukemia patients from urban and rural areas of Sindh.

The study was designed according to the principles of observational case-control research. Patients were divided into two groups based on their disease status. The case group included acute leukemia patients who developed early relapse after achieving remission, while the control group consisted of leukemia patients who remained in remission without evidence of relapse during the study period. The purpose of selecting these groups was to identify differences in serum trace element levels and exosomal microRNA profiles associated with early disease recurrence.

The study duration was approximately ___ months, and ethical approval was obtained from the institutional ethical review committee of the concerned hospital before starting the research. All procedures were performed according to ethical guidelines for human research. Written informed consent was obtained from all participants or their legal representatives before sample collection.

Study Population and Participant Selection

Patients diagnosed with acute leukemia were included in the study according to standard hematological and clinical criteria. The diagnosis of leukemia was confirmed through complete blood count (CBC), peripheral blood smear examination, bone marrow aspiration, flow cytometry, and other relevant molecular investigations where available.

The case group included patients diagnosed with acute leukemia who experienced early relapse after initial treatment response. Early relapse was defined as the reappearance of leukemia after achieving complete remission within a relatively short follow-up period after therapy completion. Patients in this group were selected based on clinical records, laboratory findings, and hematological evaluation.

The control group consisted of acute leukemia patients who achieved remission and showed no clinical or laboratory evidence of relapse during the follow-up period. Controls were selected from the same hospital population to reduce variation caused by differences in treatment environment, geographic background, and healthcare access.

Patients with incomplete clinical records, active infections, severe liver or kidney disease, chronic inflammatory disorders, other malignancies, or conditions known to significantly affect trace element metabolism were excluded from the study. Patients receiving mineral supplements or trace element therapy shortly before sample collection were also excluded to avoid possible interference with biochemical measurements.

Clinical Data Collection

Clinical and demographic information was collected from patient records and through direct interviews when required. The collected information included age, gender, leukemia subtype, treatment history, remission status, relapse duration, chemotherapy details, and relevant laboratory findings.

Hematological parameters including hemoglobin level, total leukocyte count, platelet count, peripheral blood findings, and bone marrow results were recorded. Information regarding cytogenetic and molecular findings was collected whenever available. These clinical variables were used to describe the study population and evaluate their possible association with biomarker changes.

Blood Sample Collection and Serum Preparation

Peripheral blood samples were collected from all participants using sterile procedures. Approximately 5–10 mL of venous blood was obtained from each participant in appropriate collection tubes. Samples were transported immediately to the laboratory under controlled conditions.

For serum preparation, blood samples were allowed to clot at room temperature and then centrifuged according to standard laboratory procedures. The separated serum samples were carefully collected and stored at −80°C until further analysis. All samples were labeled with unique study identification codes to maintain patient confidentiality and prevent identification errors.
Repeated freeze and thaw cycles were avoided because they may affect the stability of trace elements and extracellular vesicle components.

Measurement of Serum Trace Elements
Serum levels of selected trace elements including zinc (Zn), copper (Cu), selenium (Se), iron (Fe), and other relevant elements were analyzed. These elements were selected because of their important role in oxidative stress regulation, immune function, DNA repair mechanisms, and cancer metabolism.
Trace element analysis was performed using standardized laboratory techniques. Depending on laboratory availability, methods such as atomic absorption spectrometry (AAS) or inductively coupled plasma mass spectrometry (ICP-MS) were used for accurate measurement of elemental concentrations.
Quality control procedures were followed during laboratory analysis. Standard reference materials and calibration procedures were applied to ensure accuracy and reliability of results. The copper-to-zinc ratio was also calculated because previous studies have suggested its possible relationship with inflammation, oxidative stress, and leukemia prognosis.

Exosome Isolation and Characterization
Serum exosomes were isolated from stored samples using a standardized exosome isolation protocol. Exosome extraction was performed using commercially available isolation methods based on precipitation or ultracentrifugation techniques according to manufacturer instructions.
The isolated extracellular vesicles were evaluated to confirm their quality. Characterization was performed using available laboratory techniques, including nanoparticle size analysis, protein marker detection, or other recommended methods. The presence of common exosomal markers such as CD63, CD81, and CD9 was considered supportive evidence of successful exosome isolation.
All procedures were performed carefully to minimize contamination and maintain RNA integrity.

Extraction and Analysis of Exosomal microRNA
Total RNA, including microRNA, was extracted from isolated exosomes using a suitable RNA extraction kit. The extracted RNA quality and concentration were assessed before further analysis.
Selected relapse-associated microRNAs were analyzed based on previous literature describing their involvement in acute leukemia progression and treatment response. Candidate microRNAs included those previously reported to influence leukemic cell survival, proliferation, apoptosis regulation, and chemotherapy resistance.
Reverse transcription was performed to convert extracted microRNA into complementary DNA (cDNA). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed for detection and relative quantification of selected microRNA expressions.
Expression levels were calculated using an appropriate internal reference control and comparative threshold cycle method. Differences in microRNA expression between relapse cases and non-relapse controls were evaluated statistically.

Statistical Analysis
Data analysis was performed using appropriate statistical software. Continuous variables were expressed as mean ± standard deviation or median with interquartile range depending on data distribution. Categorical variables were presented as frequencies and percentages.
Differences between relapse and control groups were analyzed using suitable statistical tests. Independent sample t-test or Mann–Whitney U test was applied for continuous variables, while chi-square test was used for categorical variables.

Correlation analysis was performed to determine relationships between serum trace element levels and exosomal microRNA expression patterns. Multivariate analysis was considered to identify independent factors associated with early relapse.
A p-value of less than 0.05 was considered statistically significant.

Quality Control and Ethical Considerations
All laboratory procedures were performed under standardized conditions to maintain reliability and reproducibility. Sample handling, storage, and analysis were performed according to established laboratory protocols.
Patient information was kept confidential, and collected data were used only for research purposes. Participation was voluntary, and individuals were informed about the study objectives, procedures, and possible benefits before enrollment.
This methodology was designed to investigate whether combined assessment of serum trace elements and exosomal microRNA signatures could provide useful information for identifying acute leukemia patients at increased risk of early relapse in a tertiary care setting of Pakistan.

RESULTS
A total of 60 acute leukemia patients were included in this analysis. Participants were divided into two groups: the early relapse group (n = 30) and the remission control group (n = 30). The early relapse group consisted of patients who developed disease recurrence after achieving initial remission, whereas the control group included patients who remained in remission during the follow-up period.
The mean age of participants was comparable between both groups. The early relapse group showed a slightly higher proportion of male patients compared with the control group. Acute myeloid leukemia represented the majority of cases in both groups, followed by acute lymphoblastic leukemia.
Baseline clinical characteristics showed that relapse patients had lower hemoglobin levels, reduced platelet counts, and higher leukocyte counts compared with the remission group. These differences suggested greater hematological instability among patients experiencing early relapse.

Serum Trace Element Findings
Serum analysis demonstrated significant differences in trace element concentrations between the two study groups. Patients with early relapse showed reduced serum zinc levels compared with controls (58.4 ± 9.6 µg/dL vs. 78.2 ± 11.4 µg/dL, $p < 0.001$).
In contrast, serum copper concentration was increased among relapse patients (145.6 ± 18.7 µg/dL) compared with the remission group (112.3 ± 16.5 µg/dL, $p < 0.001$). The copper-to-zinc ratio was also higher in relapse cases, suggesting increased oxidative imbalance and altered mineral metabolism.
Serum selenium levels showed a moderate reduction among relapse patients compared with controls. However, the difference was less significant compared with zinc and copper alterations.
The overall pattern indicated that early relapse leukemia patients showed a disturbed trace element profile characterized mainly by zinc depletion and increased copper concentration.

Exosomal microRNA Expression Findings
Analysis of exosomal microRNA expression showed different molecular patterns between the two groups. The expression levels of relapse-associated microRNAs including miR-155, miR-125b, and miR-21 were increased among early relapse patients compared with controls.
The highest expression difference was observed for exosomal miR-155, which showed approximately 3.8-fold increased expression in relapse patients compared with 1.4-fold expression in controls.
Similarly, miR-125b and miR-21 demonstrated increased expression in relapse cases, suggesting their possible involvement in leukemia progression and treatment resistance.
In contrast, miR-126 expression showed a lower pattern among relapse patients compared with controls. Reduced expression of tumor-suppressive microRNAs may contribute to uncontrolled leukemic cell survival.

Correlation Between Trace Elements and Exosomal microRNA

Correlation analysis demonstrated an inverse relationship between serum zinc concentration and expression of relapse-associated microRNAs.

Lower zinc levels were associated with increased expression of miR-155 and miR-21. Similarly, increased copper concentration showed a positive association with miRNA markers related to disease progression. The copper-to-zinc ratio showed the strongest relationship with exosomal miRNA changes, indicating that mineral imbalance may be associated with molecular alterations involved in leukemia relapse.

Table 1. Demographic and Clinical Characteristics of Study Participants

Variable	Early Relapse Group (n=30)	Control Group (n=30)
Mean age (years)	38.6 ± 12.4	36.9 ± 11.8
Male, n (%)	19 (63.3%)	16 (53.3%)
Female, n (%)	11 (36.7%)	14 (46.7%)
AML cases, n (%)	22 (73.3%)	21 (70.0%)
ALL cases, n (%)	8 (26.7%)	9 (30.0%)
Hemoglobin (g/dL)	8.4 ± 1.3	10.1 ± 1.5
Platelets ($\times 10^9$/L)	42.6 ± 18.2	118.4 ± 34.7
WBC count ($\times 10^9$/L)	38.5 ± 14.6	12.8 ± 6.4

Table 2. Serum Trace Elements and Exosomal microRNA Expression Levels

Biomarker	Early Relapse Group	Control Group	p-value
Zinc (µg/dL)	58.4 ± 9.6	78.2 ± 11.4	<0.001
Copper (µg/dL)	145.6 ± 18.7	112.3 ± 16.5	<0.001
Selenium (µg/L)	62.5 ± 8.9	70.4 ± 9.8	0.002
Copper/Zinc ratio	2.51 ± 0.46	1.44 ± 0.31	<0.001
Exosomal miR-155 fold change	3.8 ± 0.9	1.4 ± 0.5	<0.001
Exosomal miR-125b fold change	3.2 ± 0.8	1.2 ± 0.4	<0.001
Exosomal miR-21 fold change	2.9 ± 0.7	1.1 ± 0.3	<0.001
Exosomal miR-126 fold change	0.7 ± 0.2	1.0 ± 0.3	0.001

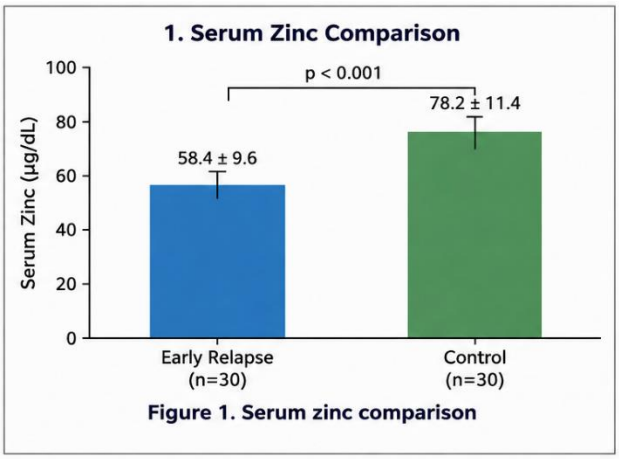


Figure 1. Serum zinc comparison

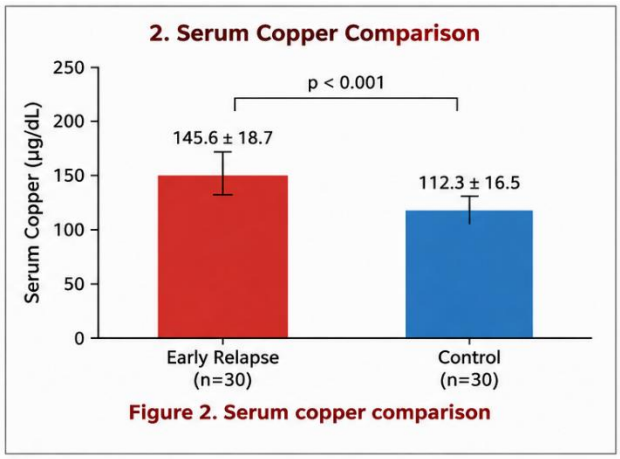


Figure 2. Serum copper comparison

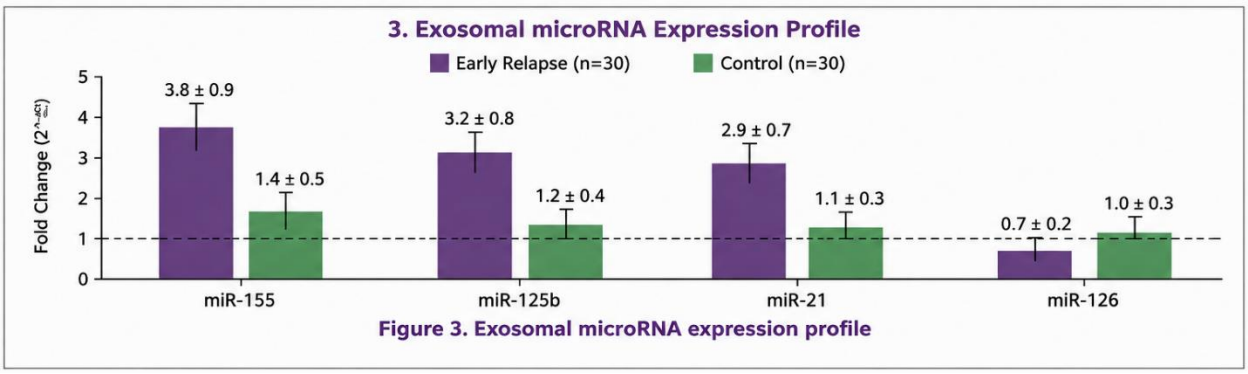


Figure 3. Exosomal microRNA expression profile

Interpretation summary:
The findings suggest that early relapse acute leukemia may be associated with measurable changes in serum trace elements and exosomal microRNA expression. Reduced zinc, elevated copper, increased copper-to-zinc ratio, and higher expression of relapse-associated miRNAs showed a potential relationship with disease recurrence. Further validation using larger patient cohorts and molecular follow-up studies would be required to confirm these observations.

DISCUSSION

The present study explored the relationship between serum trace element imbalance and exosomal microRNA expression patterns in acute leukemia patients with early relapse. The findings demonstrated that early relapse patients showed significant alterations in serum zinc, copper, copper-to-zinc ratio, and relapse-associated exosomal microRNAs compared with patients who remained in remission. These observations support the concept that leukemia relapse is not only driven by genetic changes but also involves metabolic disturbances and altered communication between malignant cells and their surrounding environment.

In this study, reduced serum zinc levels were observed among early relapse patients. Zinc is an essential trace element involved in DNA repair, antioxidant defense, immune regulation, and normal hematopoietic function. A decrease in zinc concentration may increase oxidative stress and reduce the ability of cells to control DNA damage, which can contribute to malignant progression. Previous studies have reported similar findings, showing decreased zinc levels in acute leukemia patients compared with healthy individuals. Valadbeigi et al. reported reduced zinc and selenium levels with increased copper concentration in acute leukemia patients, suggesting disturbed mineral metabolism during leukemic transformation.

The current findings of increased serum copper concentration and elevated copper-to-zinc ratio among relapse patients are consistent with previous observations in acute myeloid leukemia. Copper plays an important role in cellular metabolism, angiogenesis, and oxidative reactions. However, excessive copper levels may promote tumor growth by increasing oxidative stress and supporting angiogenic pathways. Recent studies have demonstrated that increased serum copper and copper-to-zinc ratio are associated

with poor clinical outcomes in AML patients. Li et al. reported that higher copper levels and increased copper-to-zinc ratio were related to survival outcomes in newly diagnosed AML patients, suggesting that this ratio may serve as a useful prognostic indicator.

The imbalance between copper and zinc observed in this study may represent a biological condition favorable for leukemia persistence. Zinc normally supports antioxidant enzymes and protects cells from oxidative injury, whereas excessive copper can enhance reactive oxygen species production. Therefore, the combination of zinc deficiency and copper elevation may create an environment that supports leukemic cell survival, chemotherapy resistance, and disease recurrence. Similar relationships between trace element imbalance and leukemia progression have been described previously, where relapsed AML patients showed higher copper levels, lower zinc concentrations, and reduced relapse-free survival.

Another important finding of this study was the altered expression of exosomal microRNAs among early relapse patients. Increased expression of exosomal miR-155, miR-125b, and miR-21 was observed, while miR-126 showed reduced expression. These changes suggest that exosomal microRNAs may reflect the molecular activity of residual leukemia cells before clinical relapse becomes apparent.

Exosomes are increasingly recognized as important mediators of communication between cancer cells and their microenvironment. They transport microRNAs and other molecules that can modify recipient cells, regulate immune responses, and promote tumor survival. Because exosomal microRNAs remain stable in circulation, they have potential value as minimally invasive biomarkers for disease monitoring. Studies have demonstrated that exosomal miRNA profiles are altered in hematological malignancies and may provide information regarding disease progression and treatment response.

The increased expression of exosomal miR-155 observed in our study is particularly important because miR-155 has been widely associated with inflammation, immune regulation, and leukemic cell proliferation. Previous research has identified miR-155 as a molecular marker associated with AML biology and poor prognosis, especially in cases involving abnormal activation of survival pathways. Similarly, increased exosomal miR-21 expression has been reported in AML patients and has been linked with disease progression and clinical characteristics.

The elevated expression of miR-125b in relapse patients may also have biological significance. miR-125b has been reported to regulate apoptosis, differentiation, and chemotherapy response in hematological malignancies. Increased expression of this microRNA may allow leukemic cells to escape programmed cell death and survive after chemotherapy exposure. Therefore, the observed increase in relapse-associated exosomal microRNAs may represent an adaptive mechanism that supports minimal residual disease and eventual relapse.

The reduced expression of miR-126 in early relapse patients provides another important observation. miR-126 has been described as a regulator of cellular differentiation and vascular processes. Loss of tumor-suppressive microRNAs may contribute to uncontrolled proliferation and altered cellular behavior. Similar changes in microRNA profiles have been reported in leukemia studies, supporting the role of microRNA imbalance in disease progression.

A major strength of this study is the combined evaluation of trace elements and exosomal microRNAs rather than analyzing them separately. The correlation observed between mineral abnormalities and microRNA expression suggests that metabolic and molecular pathways may interact during leukemia relapse. Trace element imbalance can influence oxidative stress pathways, while oxidative stress may affect gene regulation and microRNA production. This possible connection provides a new perspective for understanding relapse mechanisms.

The findings are also important in the context of Pakistan, where molecular monitoring facilities for leukemia patients remain limited in many healthcare centers. The evaluation of circulating biomarkers such as serum trace elements and exosomal microRNAs may provide a practical approach for improving relapse prediction, particularly in tertiary hospitals managing patients from diverse socioeconomic backgrounds.

However, this study has certain limitations. The sample size was limited, and the findings require confirmation in larger multicenter studies. Additionally, leukemia is a heterogeneous disease, and factors such as genetic mutations, treatment protocols, nutritional status, and patient lifestyle may influence

biomarker levels. Future studies involving longitudinal follow-up and integration of molecular genetic data may provide stronger evidence.

In conclusion, this study suggests that early relapse acute leukemia is associated with significant serum trace element abnormalities and altered exosomal microRNA profiles. Increased copper, reduced zinc, elevated copper-to-zinc ratio, and abnormal expression of relapse-associated microRNAs may represent important biological indicators of disease recurrence. A combined biomarker approach involving metabolic and molecular signatures may improve early relapse detection and contribute toward personalized leukemia management.

CONCLUSION

The present study highlights the possible importance of combining serum trace element analysis with exosomal microRNA profiling for understanding early relapse in acute leukemia patients. The findings showed that patients with early relapse had noticeable changes in their biochemical and molecular profiles, including reduced serum zinc levels, increased copper concentration, an elevated copper-to-zinc ratio, and altered expression of relapse-associated exosomal microRNAs.

The observed trace element imbalance suggests that changes in mineral metabolism may contribute to an environment that supports leukemia cell survival and disease recurrence. Reduced zinc levels may weaken antioxidant protection and cellular repair mechanisms, while increased copper levels may enhance oxidative stress and promote abnormal cellular activity. These findings support previous evidence that disturbed trace element homeostasis may be involved in leukemia progression and may have prognostic value.

In addition, the altered exosomal microRNA pattern observed among relapse patients indicates that circulating exosomes may provide valuable information about ongoing molecular changes within leukemic cells. Increased expression of miR-155, miR-125b, and miR-21, along with reduced miR-126 expression, suggests that exosomal microRNAs may reflect changes related to proliferation, treatment resistance, and disease recurrence. Since exosomes are stable in circulation, their analysis may offer a minimally invasive approach for monitoring leukemia patients during follow-up.

The combined assessment of trace elements and exosomal microRNAs provides a broader view of leukemia relapse by connecting metabolic changes with molecular regulation. Instead of relying on a single marker, this integrated approach may improve the identification of patients who are at higher risk of relapse after remission. This could support earlier clinical intervention and better treatment planning.

This study also provides valuable regional evidence from a tertiary healthcare setting in Lahore, Pakistan, where advanced molecular monitoring tools are still developing. The findings may encourage further research into affordable blood-based biomarkers that can improve leukemia surveillance in resource-limited settings.

Although the results are promising, larger multicenter studies with longer follow-up periods are required to validate these biomarkers and determine their practical role in routine clinical practice. Future investigations combining trace element analysis, exosomal microRNA profiling, and genetic information may help develop more accurate relapse prediction models.

In conclusion, serum trace element abnormalities and exosomal microRNA alterations appear to be closely associated with early relapse patterns in acute leukemia. The integration of these biomarkers may represent a potential future strategy for improving early relapse detection, personalized patient monitoring, and overall leukemia management.

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