The expression level of aspartate beta-hydroxylase (ASPH) and clinical importance in acute myeloid leukemia

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Background
Acute myeloid leukemia (AML) is a heterogeneous disorder characterized by clonal expansion of myeloid progenitors in the bone marrow and peripheral blood. Aspartate β-hydroxylase (ASPH) is a membrane protein that promotes cellular motion. Recent studies have revealed that ASPH is an indicator of carcinoma in humans and is highly expressed in AML.

Patients and methods
The study included 30 AML patients diagnosed and classified by complete blood count with differential, bone marrow study, flow cytometric, cytogenetic, and molecular studies, and 10 normal participants of the same age group. The serum level of ASPH was measured in both cases and controls by enzyme-linked immunosorbent assay.

Results
The level of ASPH was significantly higher in patients than in controls ($P < 0.001$). Patients with mutant FLT3 had higher serum ASPH compared with those with wild FLT3 with a trend toward statistical significance ($P = 0.074$). Other clinical and laboratory variables had no relationship with ASPH levels in the blood.

Conclusion
This study detected a significant increase in ASPH levels in the blood of AML patients more than the controls, shedding the light on the role of ASPH in AML genesis and paving the way for the development of new medications that block ASPH's leukemogenesis effect in the future.

Keywords:
adult, acute myeloid leukemia, aspartate β-hydroxylase, enzyme-linked immunosorbent assay

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Introduction

Acute myeloid leukemia (AML) is characterized by clonal development of myeloid progenitors in the bone marrow and peripheral circulation (AML). Thanks to advancements in treatment regimens and supportive care, it is curable now in ∼35–40% of young patients under the age of 60 years [1]. The outlook for people over 60 years has improved. However, the situation remains gloomy [2]. Most of the adults with de novo AML have chromosomal abnormalities (deletions and translocations) known as the promoters of AML. Cytogenetic anomalies t(8;21)(q22;q22), t(15;17)(q22;q12), and inv (16)(p13.1;q22) are linked to a long remission and a better chance of survival, but complex karyotyping and 11q23 are linked to shorter survival [1]. Alternatively, 40–50% of cases have normal cytogenetics (CN-AML) [3]. Even though this particular group has a medium chance of relapsing, there is a considerable amount of heterogeneity regarding their prognosis. This type of AML requires extensive molecular testing for risk stratification and therapy decisions [4]. The identification of new mutations in AML has raised prognostic and probably therapeutic implications.

Although treatment has improved considerably in recent years, AML remains difficult to cure with a high relapse rate. Relapsed/refractory AML patients who are elderly or unfit for cytotoxic chemotherapy and whose disease does not respond to hypomethylating agents represent a major challenge in their treatment [5]. Aspartate β-hydroxylase (ASPH is a transmembrane protein that hydroxylates aspartyl and asparaginyl residues within EGF-like domains), promoting cell motility, migration, and adhesion. It is highly expressed in developing embryos, but not in any other healthy adult human tissue [5].

High expression of ASPH in humans was identified as an indication of cancer in humans as early as 1996. In addition, research has linked elevated ASPH levels (variously in the affected tissue or blood serum) with hepatocellular carcinoma [6,7], adenocarcinoma (pancreatic cancer) [8], prostate cancer [7], and lung cancer [9]. The pancreatic study [8]

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showed elevated ASPH only in the diseased tissue, but not in the adjacent normal and inflamed tissue.

40% of AML patients over express ASPH, which could be a promising therapeutic target. An ASPH nanoparticle vaccination for solid tumors has demonstrated encouraging outcomes. There are several strategies in the work to expand clinical testing of ASPH targeting for AML [5].

Aim

To determine the level of ASPH expression in the serum of Egyptian AML patients and to investigate its clinical implications.

Patients and methods

Study design

Cross-sectional.

Study population

This study involved 30 patients and 10 age-matched and sex-matched healthy controls attending the Hematology Clinic, Ain Shams University between August 2021 and March 2022.

Patients were diagnosed by complete blood count, bone marrow aspiration, flow cytometry, cytogenetics, and molecular analysis. Inclusion criteria: age 16 years or more, newly diagnosed, and primary or secondary to MPN or MDS were included. Patients under 16 years, secondary to solid tumors or pregnant females were excluded. This study was conducted by the guidelines approved by the ethics committee of Ain Shams University, Egypt. All participants in the study gave their written informed permission.

Specimen collection and evaluation

Whole blood was collected from patients in EDTA tubes for complete blood count and blood film; bone marrow aspiration was collected in EDTA tubes to perform flow cytometry, cytogenetic and molecular study analysis, besides bone marrow film staining and examination. Cerebrospinal fluid was drawn and examined in certain cases M4 and M5. Coagulation profiles were drawn in sodium citrate tubes. Chemistry and ASPH assay samples were drawn in serum tubes. ASPH assay using the enzyme-linked immunosorbent assay Kit 'Human Aspartyl/Asparaginyl Beta-hydroxylase,' Bioassay Technology Laboratory Cat. No E4537Hu.

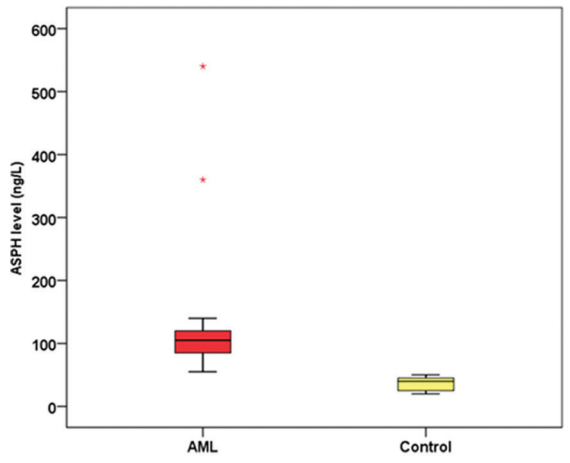
Statistical analysis

Statistical analysis was done using IBM SPSS Statistics, version 23 (IBM Corp., Armonk, New York, USA). The numerical information was expressed as median and range or mean and SD. Frequency and percentage were used to express qualitative data. The Mann–Whitney test was used to compare quantitative data between two groups. The Spearman-rho method was used to see the correlation between numerical variables. A P value less than 0.05 was considered significant.

Results

This study had 40 participants, 30 patients and 10 age-matched controls. The patient characteristics are presented in Table 1. Serum ASPH median level in the patients was 105 ng/l (range: 55–540 ng/l) and in the control group it was 40 ng/l (range: 20–50 ng/l; $P < 0.001$) (Fig. 1). The bulk of the patients had an FAB class (M2), with only six M4 patients. AML was secondary in four patients. In most of the cases ($n = 24$), cytogenetics was normal. FMS-like tyrosine kinase 3 internal tandem duplication (FLT3-ITD) was discovered in seven AML patients (Table 1). CNS infiltration was not detected in any patient. No evident link between ASPH serum levels and FAB categorization was detected. Statistical comparison was only possible between M1 + M2 and M4 + M5. There was no significant difference between these two groups ($P = 0.417$) (Table 2). Between patients with and without extramedullary disease, there was no significant difference in serum ASPH levels ($P = 0.439$) (Table 3). Patients with normal and abnormal cytogenetics had similar serum levels of ASPH ($P = 0.601$) (Table 4). No significant difference was detected between high-risk to low-risk and

Figure 1



ASPH levels in AML and control groups. P value less than 0.05 is taken as significant. AML, acute myeloid leukemia; ASPH, aspartate β-hydroxylase.

Table 1 Clinical and laboratory data of the acute myeloid leukemia group (*n*=30)

Variables	Finding
Age	49.2±16.0
Sex [*n* (%)]	
Male	18 (60)
Female	12 (40)
Clinical characteristics	
Hypertension	3
Diabetes mellitus	2
Cardiac	1
Hepatic	1
Extramedullary disease [*n* (%)]	
Liver	4 (13.3)
Spleen	8 (26.7)
Lymph nodes	7 (23.3)
Hemoglobin (g/dl)	4.4-10.6
Total leukocyte count (×10^3/mm^3)	0.5-153.0
Platelet count (×10^3/mm^3)	12.0-237.0
Peripheral blood blasts (%)	4-88
Bone marrow blasts (%)	20-96
Lactic acid dehydrogenase (IU/L)	149-1006
Uric acid	2.4-5.6
FAB classification [*n* (%)]	
M2	15 (50)
M4	6 (19.9)
Other	9 (21.1)
Secondary	6
Cytogenetics [*n* (%)]	
Normal	24 (80)
t (9;22)	3 (10)
t (8;21)	2 (6.7)
Complex	1 (3.3)
FLT3-ITD	7 (23.3)

Table 2 Level of serum aspartate β-hydroxylase in relation to FAB classification of the acute myeloid leukemia group

	N	Median	Range	*P*
M1+M2	19	95	60-540	0.417
M4+M5	7	120	55-140	

P value less than 0.05 is taken as significant.

Table 3 Level of serum aspartate β-hydroxylase in relation to presence of extramedullary disease in the acute myeloid leukemia group

	N	Median	Range	*P*
Extramedullary disease	9	110	65-540	0.439
No extramedullary disease	21	100	55-360	

P value less than 0.05 is taken as significant.

Table 4 Level of serum aspartate β-hydroxylase in relation to cytogenetic status of the acute myeloid leukemia group

	N	Median	Range	*P*
Normal	24	115	55-540	0.651
Abnormal	6	90	70-140	

P value less than 0.05 is taken as significant.

intermediate-risk cytogenetic categories related to the serum level of ASPH (P = 0.334) (Table 5). Patients with mutant *FLT3* had higher serum ASPH compared with those with wild *FLT3*. There was a trend toward statistical significance between mutant and wild groups (P = 0.074) (Fig. 2).

Discussion

AML is a heterogeneous disease with a very variable prognosis and a high mortality rate: 5-year overall survival is lesser than 50%, and in elderly patients, only 20% will survive 2 years after diagnosis [10].

Due to the heterogeneity of AML, a panel of biomarkers seems to be more appropriate from a clinical perspective. Therefore, finding a pattern of multiple biomarkers can provide crucial diagnostic and prognostic information. In terms of risk stratification, genetic and cytogenetic alterations are markers of great importance for clinical management [11].

Protein research can also help with risk classification, which can lead to the development of a more effective, targeted and less toxic therapy. Because proteins are directly responsible for cell function, aberrant protein expression indicates cellular disruption as a result of a pathogenic situation [12]. A recent study has revealed the overexpression of ASPH in 40% of AML patients, suggesting a possible therapeutic target. Furthermore, they stated that the ASPH nanoparticle vaccine is now being studied in clinical trials and has demonstrated good outcomes in solid tumors [5].

The current study found that AML patients' ASPH levels were considerably greater than the control group (P < 0.001). The minimal level in AML patients was higher than the maximum level detected in controls (55 and 50 ng/l, respectively). A previous study has investigated the usefulness of ASPH gene expression levels as a marker for minimum residual disease in AML. Real-time quantitative PCR examination of leukocytes from fresh whole blood obtained at diagnosis, and the treated patients who achieved CR were compared with healthy controls to measure ASPH expression levels. The authors found that the patients displayed about an 8.6-fold increased expression of the ASPH transcript at diagnosis compared with healthy controls. On average, ASPH expression decreased to essentially normal levels in treated patients (n = 27). They advocated ASPH expression as a molecular marker for AML that may be helpful in monitoring remission and detecting relapse [13]. A more recent study [5] collected bone marrow aspirate (n = 32) and peripheral blood samples (n = 10) from AML patients.

They used flow cytometry to examine the samples and discovered ASPH myeloblast expression in 40%

Table 5 Level of serum aspartate β-hydroxylase in relation to cytogenetic risk of the acute myeloid leukemia group

	N	Median	Range	*P*
Low	2	90	85-95	0.304
Intermediate	19	100	55-360	
High	9	110	70-540	

P value less than 0.05 is taken as significant.

of them, using a mean fluorescence intensity of 10 as a criterion for positive ASPH surface expression. In nonneoplastic cells, ASPH expression was not detected, such as CD34+ hematopoietic stem cells, B- or T-lymphocytes, or monocytes.

The control group had a low level of ASPH by the previous findings of low or absent ASPH in the normal population [6,14–16].

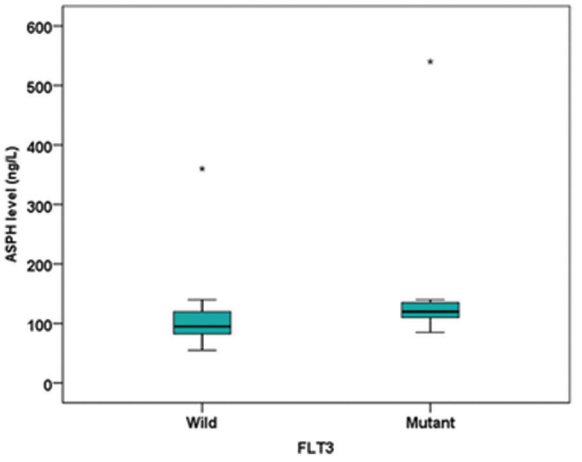
High serum ASPH expression did not seem to be associated with the patients' clinical or laboratory prognostic variables in the current investigation. It had no relation with FAB classification (P = 0.417). There was no significant difference in ASPH serum levels between patients with and without the extramedullary disease (P = 0.439). Similarly, ASPH was not associated with abnormal cytogenetics (P = 0.601) or cytogenetic risk (P = 0.334). The amount of serum ASPH did not affect treatment response at day 28 (P = 0.344). These findings are concordant with that of Lebowitz *et al.* [13] and Holtzman *et al.* [5]. The only statistically significant connection between FLT3 mutation and increased serum ASPH (P = 0.074) was a trend. The relation between high ASPH levels and FLT3 mutation in the current study may indicate a possible prognostic potential of ASPH, which can be studied in future large-scale studies. FLT3 gene mutations are known to occur in ~30% of all AML cases, with the internal tandem duplication being the most common type of FLT3 mutation (FLT3-ITD). It is a common driver mutation in AML patients that causes a significant leukemic load and has a bad prognosis [17].

The present research includes a few flaws. The sample size was small and single-centered. To infer the presence of a substantial relationship between serum ASPH and AML patients, future multicenter research with large sample sizes is required.

Conclusions

ASPH levels should be evaluated in the risk assessment of de novo AML patients. It has the potential to be used as a molecular marker for AML that may be used to track remission and relapse. There exists a weak but substantial link between FLT3 mutation and the high expression of ASPH. Inhibition of ASPH would almost certainly modify patients' prognosis. Future research should focus on discovering new medications that target the ASPH gene.

Figure 2



Serum ASPH about FLT3 mutation in the AML group. *P* value less than 0.05 is taken as significant. AML, acute myeloid leukemia; ASPH, aspartate β-hydroxylase.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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