Research Paper

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Prognostic Value of Human Chitinase 3-Like 1 in Adult Acute Myeloid Leukaemia

Medical Science

KEYWORDS : Acute myeloid leukemia (AML),Human Chitinase 3-like-1(YKL40), Survival

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ABSTRACT

Human Chitinase 3-like-1(YKL40) secreted by cancer cells, macrophages, and neutrophils, plays role in angiogenesis, differentiation and metastasis also associated with poor prognosis in solid carcinomas,short recurrence-free interval and short overall survival. The aim of this workwas toevaluatethe role of human Chitinase 3-like 1 in adult patients with acute myeloid leukemia and its relation to other prognostic factors and treatment response.Patients and methodsThis study conducted with eighty six de novo AML patients their age ranged 18 to 60 years with median age thirty seven years and serum human Chitinase 3-like-1(YKL40)concentration was determined by a two-site, sandwich-type ELISA.Results: The results of this study revealed thatserum YKL40 was high in seventy cases (81.4%)andsignificant association with treatment response was detected (p< 0.05),a significant relation between the mean value ± S.D of serum level of YKL40 in acute myeloid leukemia patientsand the apparently healthycontrol was detected, also before and after chemotherapy and responders who achieved complete remission, however there was increment of the mean value of serum YKL-40 after failure of remission induction(Non responder) with insignificant p value (0.799).overall survival in patients with high initial levels of serum YKL-40 was significantly less than those with normal serum levels of YKL40 (P<0.03) and disease free survival was significantly higher in patients with initially normal serum levels of YKL-40 with p value < 0.05 and there was also a highly significant relation between disease free survival and overall survival in those responders to treatment and Non responders.ConclusionHuman Chitinase 3-like-1was a novel biomarker for predicting outcome and survival in acute myeloidleukemia patients and normalization of initially high plasma YKL-40 was an important sign of response.

Introduction

Acute myeloid leukemia (AML) is a malignant disorder of the blood characterized by impaired differentiation and proliferation of hematopoietic precursor cells, resulting in abnormal accumulation of immature precursors and suppression of growth and maturation of cells involved in normal hematopoiesis (**1**)

The prognosis of acute myeloid leukemia (AML) patients is very variable, ranging from survival of a few days to cure and clinical outcome can be partly predicted by age, cytogenetic findings, and serum lactate dehydrogenase at the time of diagnosis. However, the prognosis of an individual AML patient cannot yet be estimated accurately. It is therefore important to identify new biomarkers in acute myeloid leukemia patients for the prediction of prognosis, treatment response, detection of relapse, and monitoring for minimal residual disease. (**2**)

Human Chitinase 3-like-1(CHI3L1), also named human cartilage glycoprotein-39 (HC gp-39), 38-KDa heparin binding glycoprotein (Gp38k), breast-regressing protein 39 KDa (brp-39), and Chondrex secreted by cancer cells, macrophages, and neutrophils. It might be a growth or differentiation factor, played a role in angiogenesis, differentiation and metastasis (**3**)

High serum human Chitinase 3-like-1 (YKL-40)associated with poor prognosis in solid carcinomas. Several studies of patients with solid tumors had shown that serum YKL-40 elevated in some patients with primary or metastatic carcinoma of the breast (**4**), colorectal (**5**), ovary (**6**), lung (**7**), prostate (**8**),cervical adenocarcinoma**(9)** kidney and glioblastoma (**10**). Interestingly, the studies showed that high serum YKL-40 was related to short recurrence-free interval and short overall survival, and that had a high serum YKL-40 was an independent prognostic variable of poor prognosis

Aim of the work

This study was aimed to evaluate the role of human Chitinase 3-like 1(YKL-40) in adult patients with acute myeloid leukemia and its relation to other prognostic factors and treatment response.

Patients and methods

This study was carried out at hematology and oncology unit, internal medicine department, zagazig university hospital(Zagazig, Egypt) during the period between February 2011 and June 2012. It included eighty six de novo acute myeloid leukemia patients and ten healthy volunteers , all patients were given their informed consent after approval of our local ethical committee and all patients had severe cardiac, pulmonary, hepatic, renal, neurological, metabolic disease, concomitant malignancies or uncontrolled infections were excluded from the study and patients with acute promyelocytic leukemia were excluded as they had a different natural history and different management.

All patients were subjected to complete clinical his tory and physical examination ,routine laboratory investigations (Complete Blood Picture, Liver & kidney Functions, Serum electrolytes ,PT/INR, PTT ,Viral marker(HBs Ag, HCV Ab, HIV Ab),Bone Marrow Aspiration and biopsy, Immunophenotyping and Cytogenetic study).Chest X-ray was done and CT chest if indicated. Pelvi-abdominal ultrasonography, CT abdomen& pelvis, Electro-Cardiograph (E.C.G) and echocardiograph were done also.

Serum levels of Human Chitinase 3-like 1 was assessed before induction chemotherapy and after remission and all these patients were treated by induction 3&7 chemotherapy protocol (3 days of daunorubcine 45mg/m^2 and 7 days of continuous infusion of cytarabine 100mg/m^2)Bone Marrow evaluation was carried out at day 14 of end of induction protocol and response to treatment was evaluated according to revised recommendations of the international working group for standardization of response criteria ,Complete Remission (CR) was defined when the cellularity of the bone marrow (BM) after regeneration was near normal with <5% blast cells, the peripheral blood recovered completely, and no extra-medullary leukemic infiltrates were present.

When the bone marrow blast cell count remained between 5 and 25% but was reduced by at least 50% in comparison to the initial value, and the peripheral blood levels recovered completely, a patient will be considered to be in Partial Remission (PR). Failure to attain CR or PR will be consistent with Failure or

No response .(11). According to cytogenetic studies ,all patients were classified into favorable, unfavorable, and intermediate risk ad patients with favorable cytogenetic were challenged for consolidation high dose chemotherapy regimens as HAM i.e. High dose Ara C 1.5gm/m2 D1-3, Mitoxantone 10mg/m2 D3-5 for four cycles and then follow up. However those with unfavorable cytogenetic or intermediate were referred for arrangement for stem cell transplantation

METHOD

Serum samples of YKL-40 were collected immediately before the start of treatment, and after remission using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes and samples were stored at -80 C until analysis for YKL-40,samples from each patient were analyzed in the same assay without knowledge of the clinical, biochemical, or survival data, samples required a 50-fold dilution (10 ul of sample pulse 490 ul of Calibrator Diluent RD5P (1X).Serum concentrations of YKL-40 were determined by a two-site, sandwich-type ELISA using streptavidin- coated micro plate wells, a biotinylated-Fab monoclonal capture antibody, and an alkaline phosphatase–labeled polyclonal detection antibody. The sensitivity of the ELISA was 10 ng/L. The intra and extra assay coefficient of variation were <3.6% and <7.1%, respectively.**(12). (13)**

STATISTICAL ANALYSIS

Data was analyzed using IBM SPSS advanced statistics version 20 (SPSS Inc., Chicago, IL). Numerical data of scores were expressed as mean and standard deviation or median and range as appropriate. Chi-square test (Fisher's exact test) was used to examine the relation between qualitative variables. For not normally distributed quantitative data, comparison between two groups was done using Mann-Whitney test (non-parametric t-test). Spearman-rho method was used to test correlation between numerical variables. Survival analysis was done using Kaplan-Meier method and comparison between two survival curves was done using log-rank test. Cox-regression method was used to test the relation between numerical variables and survival. Odds ratio (OR) with it 95% confidence interval (CI) were used for risk estimation. The Receiver Operating Characteristic (ROC) curve was used for prediction of cut off values and p-value < 0.05 was considered significant.

RESULTS

Table(1): Patient characteristics of studied acute myeloid patients:-

Character	No.	%	P
Age(in years): Range(18-60) Median (37)	86	100	-----
Sex: Male Female	40 46	46.5 53.5	NS
Clinical presentation: Pallor,weakness,fatigue Fever Gum hypertrophy Purpura, bleeding Lymphadenopathy Spleenomegaly Hepatomegaly Chloroma Tumour lysis syndrome, renal impairment	 36 42 6 30 2 2 6 4 4	 41 48 6 34 2 2 6 4 4	NS
Virology: Hcv Ab +ve HBsAg +ve Hcv/Hbv –ve	18 0 68	21 0 79	-------
Bone Marrow (B.M)Blasts (%): Range (22-95) X ±SD(70.55±20.85)	86	100	
Immunophenotyping: +ve myeloid markers	86	100	
FAB classification: M1 M2 M4 M5	 14 36 26 10	 16.3 41.9 30.2 11.6	NS
Cytogenetic study: unfavorable intermediate favorable Unknown	 14 50 10 12	 16.3 58.1 11.6 14	NS
Serum YKL40 1- AML patients Before therapy (57.4.±61.9ng/ml) After therapy (40.1 ± 62.4ng/ml) 2- Control (46.6 ± 47.7ng/ml)	 86 10	 100 100	 <0.001
Therapy Response CR NR	 44 42	 51 49	 <0.05

Serum YKL40 was high in seventy cases (81.4%) of acute myeloid patients group and when chi square test (χ^2) test was applied to test the significant relation among different variables (sex, immunophenotypes ,cytogenetic types, treatment response),only significant association with treatment response was detected and p value was less than 0.05.

A high significant association between the mean value ± S.D of serum level of YKL40 in acute myeloid leukemia patients included in the study and the apparently normal control was detected, also before chemotherapy and after remission and there was a significant increment of level after complete remission more than failure

Table (2) comparison between YKL40 levels in AML patients before and after induction chemotherapy as regard response:

Variable	Before therapy	After therapy	t	P value
Response CR NR	 48.6 ± 61.8 65.7 ± 62.4	10.8 ±17.6 68.1±76.2	 3.34 0.25	 <0.001 NS

When one way ANOVA was used to test significance of difference of mean ± SD of serum YKL-40 in patients with different immunophenotypes and cytogenetic types , there were not any significant relations were detected(P value 0.30,0.56 respectively)

Table (3) Comparison between serum YKL-40 levels before induction chemotherapy in different phenotypes and cytogenetic types of AML patients

Variable	Sum of Squares	Df	Mean Square	F	P value
Immunophynotypes Between groups Within groups Total	 1.40 1.46 1.60	 3 40 86	 4.66 3.76	 1.24	 0.30
Cytogenetic types Between groups Within groups Total	 8.00 1.52 1.60	 3 40 86	 2.66 3.91	 0.68	 0.56

Bivariate Spearman correlation analysis was used to test correlation between serum YKL-40 and different variable (age, LDH level, ESR, BM blasts, TLC,HB, platelet PT, SGPT, SGOT, Creatinine level, PLT count and overall survival) there was not any significant correlation between serum YKL-40 levels and these variable before induction therapy.

Table (4): Bivariate Spearman correlation analysis test be-

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tween serum YKL-40 before induction chemotherapy and age, LDH level, ESR, TLC, and BM blasts

Variable	NO	Bivariate correlation (r)	P value
Age	86	0.06	0.68
LDH	86	0.15	0.33
ESR	86	0.19	0.21
BM blast	86	0.25	0.10
TLC	86	0.22	0.22
HB	86	0.35	0.23
PLAT	86	0.16	0.29
CR	86	0.24	0.13
AST	86	0.05	0.78
ALT	86	0.18	0.24
PT/INR	86	0.30	0.06
OS	86	-0.15	0.32

*. Correlation is significant at the 0.05 level (2-tailed).

Kaplan-Meier curve was used to estimate one year disease free and overall survival of patients and the mean overall survival in patients with high initial levels of plasma YKL-40 was significantly less than those with normal plasma levels of the marker (P=0.03) and disease free survival was significantly higher in patients with initially normal serum levels of YKL-40 with p value = 0.02 and there was also a highly significant difference between disease free survival and overall survival in those responders to treatment and Non responders

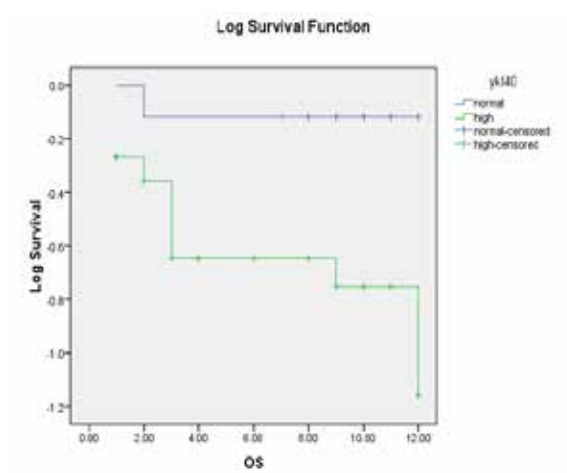

Figure (I): overall survival of patients and initial plasma YKL-40 before therapy Log rank was 4.369 and p value (0.03)was significant

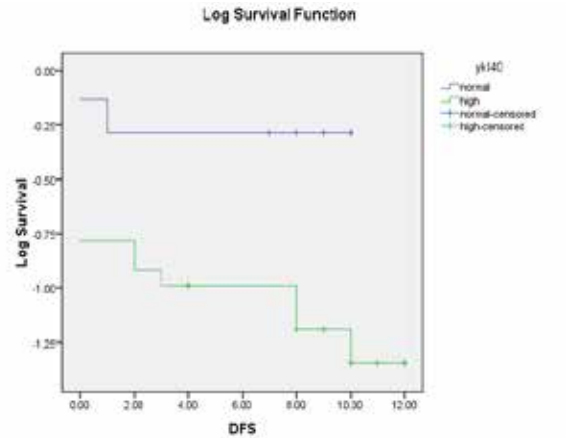

Figure (II): Disease free survival (DFS)of patients andYKL-40 before therapy Log rank was 4.694 and p value (0.03)was significant

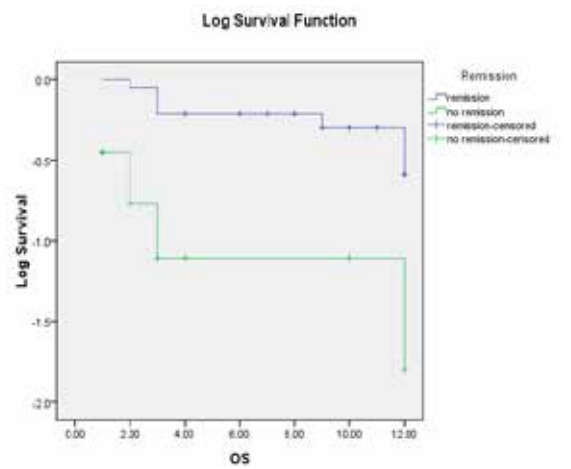

Figure (III): overall survival of patients and treatment response Log rank was 10.65 and p value (0.001) was highly significant

Discussion

Acute myeloid leukemia (AML) is a malignant hematopoietic neoplasm characterized by clonal proliferation of tumor cells that arise from the hematopoietic stem/progenitor population within the bone marrow **(14).**It is the most common acute leukemia in adults and accounts for approximately 69 percent of cases in this group AML accounts for less than 1% of all cancers and 29% of all leukemia, approximately 12,950 new cases of AML are diagnosed annually in the United States **(15)**.

The incidence of Acute myeloid leukemia (AML) increases with age, and is most frequently observed in older adults, the median age at diagnosis was 67 years **(16)** but in our study, the patient median age was 39 years and the range was 18-60 years without any significant relation between age and serum level of YKL-40 and this might attributed to inclusion criteria of patients included in the study.

The clinical signs and symptoms of AML were diverse and nonspecific, but they were usually directly attributable to the leukemic infiltration of the bone marrow, with resultant pancytopenia(17).

Fever was the most common clinical manifestation of patients in our study followed by pallor, purpuric eruption and gum hypertrophy and this was consistent with data published by Weinblatt 2004 who noted that fever and manifestations of bone marrow failure represent the most common initial clinical presentation

followed by manifestations of extra-medullary involvement.(18)

M2 of FAB morphologic classification was the most common in our study (41.5%) followed by M4 (30.4%), followed by M1 (16%).Bassan et al., 2002noted thatM1 was the commonest (27%) followed by M2 (22%) but the study of Breems et al., 2008showed that M2 was the commonest (27%) followed by M4 (23%).(19)

Cytogenetic analysis was a key component of the initial evaluation of any patient with acute myeloid leukemia (20)and this analysis of patients revealed that 16.3% of them were with unfavorable cytogenetic , 58% were with intermediate cytogenetic, 11.6% were with favorable cytogenetic and 14% were unknown. Patients with normal cytogenetic should be classified into favorable or unfavorable cytogenetic according to e.g. NPM1/ FLT3mutations in order to give more accurate data. Breems et al., 2008 noted that cytogenetic analysis of patients revealed that 34% of them were with unfavorable cytogenetic type, 29% were with intermediate cytogenetic type, 8% were with favorable cytogenetic type and 29 % were unknown and this might attributed to different selection criteria of both studied groups (19).

The biological functions of YKL-40 in hematologic malignancies and solid carcinoma were unknown and it had been suggested that YKL-40 might played a role in the proliferation and differentiation of malignant cells, protected the cells from undergoing apoptosis, stimulated angiogenesis, and had an effect on extracellular tissue remodeling, although in vivo proof of this was yet to be obtained. In vitro studies of glioblastoma cell lines have shown that diverse types of stress resulted in YKL-40 mRNA and protein expression, suggesting an involvement of YKL-40 as a cellular survival factor.(21),(22)

Serum level of YKL-40 in eighty six acute myeloid leukemia patients (excluding promyelocytic leukemia) and ten apparently healthy volunteers were measured and the median serum concentration of YKL-40 just before the start of chemotherapy in the newly diagnosed AML patients was 57.39 ng/ml (range 36.7-227.5) and was significantly higher compared with the levels in healthy volunteers (46.6 ng/ml, with range from 12.9 to170 and $P < 0.001$). according to Olav J et al 2005the median serum concentration of YKL-40 just before the start of chemotherapy in the 77 newly diagnosed AML patients was 11.2 ng/ml (range 15.3-63.7 ng/ml) and was significantly higher compared with the levels in 245 healthy volunteers (43 ng/ml, range (20-184) and $P < 0.001$)and this was nearly consistent with our data.(23)

Seventy (81%) of the AML patients had serum YKL-40 level above the upper 95th percentile of serum YKL-40 in the controls while reports of Olav J et al 2005(52%) of the AML patients had serum YKL-40 level above the upper 95th percentile (age-corrected) and this might attributed to selection criteria of studied group

Patients with elevated serum YKL-40 was an independent risk factor in acute myeloid leukemia patients without any correlation with other variable.(24)

Forty four patients (51%) had achieved remission with significant relation between serum levels of YKL-40 and achievement of complete response. However, there was not any significant relation of initial serum YKL-40 before chemotherapy in those who did not achieved remission while those had high levels were usually associated with poor outcome.

Olav J et al 2005 had noticed that serum of YKL-40 of thirty-eight patients(49%) achieved complete remission within the first four weeks and there was a significant relationship between serum YKL-40 and effect of treatment within the first months (complete remission versus non-response, Fisher exact test, $P = 0.26$ and the logistic regression for complete remission versus non responder showed a significant association with remission (odds ratio, 1.4; 95% confidence interval (CI), 1.0-1.9; $P = 0.05$ and this was matshed with our results.

Serum YKL-40 was measured in all those patients after induction chemotherapy and there was marked significant decrement of YKL-40 level in those had a high initial plasma levels and succeeded to achieve complete remission , however there was no change or even increment of serum YKL-40 levels after failure to achieve remission in Non responders, so post induction serum YKL-40 was a good indicator or monitor for response in acute myeloid leukemia patients and serum levels after induction parallel treatment outcome .

Serum level of YKL-40 before induction was significantly associated with poor survival outcome and disease free survival was significantly inversely related to initial serum YKL-40levels and univariante analysis of serum YKL-40 showed a significant association with survival within the first month and the first year after starting chemotherapy (one year survival: HR, 1.6 and $P < 0.0001$) and with the overall survival (HR, 1.3 and $P < 0.0001$).(23)

However disease free survival and overall survival was greatly related to achievement of remission and all these data had proved a role of YKL-40 as a predictor of overall and disease free survival in de novo acute myeloid leukemia patients(24)

Conclusion

YKL-40 was a novel biomarker for predicting outcome and survival in acute myeloid leukemia patients and normalization of initially high plasma YKL-40 was an important sign of response.

Disclosure

There was no conflict to author

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