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Prognostic value of Serum Levels of Progranulin in Egyptian Acute Myeloid Leukemia Patients

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Abstract

Background: *Cancer cells have defects in regulatory mechanisms that usually control cell proliferation and homeostasis. Different cancer cells share crucial alterations in cell physiology, which lead to malignant growth. Tumorigenesis or tumor growth requires a series of events that include constant cell proliferation, promotion of metastasis and invasion, stimulation of angiogenesis, evasion of tumor suppressor factors, and avoidance of cell death pathways. All these events in tumor progression may be regulated by growth factors produced by normal or malignant cells.*

Aim of the Work: *Measuring level of Progranulin (Pgrn) in the serum of adult patients with acute myeloid leukemia and correlating it with prognosis and clinical outcome.*

Materials and Methods: *This study was conducted on 80 subjects (40 patients and 40 age and sex matched healthy subjects) who were attendants to Clinical Hematology/Oncology Unit, Internal Medicine Department, Ain Shams University during the period from June 2018 to June 2019.*

Results*: We evaluated the prognostic value of progranulin in AML patients using the mean Progranulin level of 346 pg/ml to define two patient subgroups with low- versus high level. After a mean duration of follow up of 140 days (7-392) for our patients, Kaplan-Meier analyses revealed shorter overall survival (OS) and disease free survival (DFS) times in the Progranulin high versus low patient subgroups but this was statistically insignificant in case of overall survival. However, there was a tendency towards significance in case of disease free survival.*

Conclusion: *Serum progranulin level was significantly higher in AML patients than in controls (P=0.001). Progranulin level is high in AML and has high sensitivity and specificity as a diagnostic marker for AML.*

Keywords: *Progranulin - Myeloid Leukemia*

Introduction

The growth factor (GF) Progranulin (Pgrn) has significant biological effects in different types of cancer. This protein is a regulator of tumorigenesis because it stimulates cell proliferation, migration, invasion, angiogenesis, malignant transformation, resistance to anticancer drugs, and immune evasion (1).

In the extracellular matrix, Pgrn binds to receptors resulting in either activation of a signal transduction pathway or engulfment into the cell. Several studies have shown Pgrn involvement in the binding of Sortilin (SORT1) which promotes tumor cell proliferation, migration and survival, and induces drug resistance (2).

Pgrn activity is associated with p44/42 mitogen-activated protein kinase as well as phosphatidyl-inositol 3-kinases (PI3K) signaling pathways. In addition, Pgrn may stimulate the formation of the tumor stroma. Tumor necrosis factor (TNF) and Ephrin type-A receptor 2 (EPH receptor A2) were suggested as potential Pgrn facilitators (3).

In breast cancer, Pgrn has been implicated in tumorigenesis and resistance to anti-estrogen therapies for estrogen receptor positive (ER+ve) breast cancer. Previous pathological studies showed that Pgrn is expressed in invasive ductal carcinoma (IDC), but not in normal mammary epithelial tissue, benign lesions or lobular carcinoma (4).

In Rheumatoid Arthritis patients, the levels of circulating serum Pgrn have been measured and found to be significantly higher than those in age-matched healthy controls (1).

High Pgrn plasma levels were found to be strongly associated with adverse risk factors in chronic lymphocytic leukemia (CLL) patients, including unmutated Immunoglobulin heavy chain variable region (IGHV) status, expression of CD38 and ZAP-70, and poor risk cytogenetics (11q-, 17p-) suggesting that Pgrn is a novel, robust and independent prognostic marker in CLL (5).

Pgrn levels were significantly higher in the serum of patients with lymphoid malignancies than in healthy controls. High serum Pgrn levels were associated with poor prognosis in patients with diffuse large B cell lymphoma (DLBCL) (6).

Aim of the Work

The aim of the study is to measure level of Pgrn in the serum of adult patients with acute myeloid leukemia and to correlate it with prognosis and clinical outcome.

Materials and Methods

This study was conducted on 80 subjects (40 patients and 40 age and sex matched healthy subjects) who were attendants to Clinical Hematology/Oncology Unit, Internal Medicine Department, Ain Shams University during the period from June 2018 to June 2019.

Subjects:

- Eighty subjects included 40 patients diagnosed as AML by:
- Full history taking (emphasizing on symptoms of anemia, recurrent infections and bleeding per orifices).
- Thorough Clinical examination (emphasizing on lymphadenopathy, organomegaly and signs of tissue infiltration).
- Complete blood count by Coulter machine plus stained blood film.
- Routine metabolic profile; Kidney & Liver functions tests, LDH.
- Coagulation profile: Prothrombin Time& Partial Thromboplastin Time.
- BM aspiration (stained smears by leishman/Giemsa) with or without trephine biopsy (stained by H&E) for morphologic examination.
- Immunophenotyping by immunohistochemistry or flowcytometry using BD Accuri Flowcytometer (6 colors).
- Bone marrow cytogenetic studies and Molecular studies.
- Radiographic studies for assessment of extramedullary disease.
- CerebroSpinal Fluid examination (Cytology) in case of FAB M4-5 subtypes or if signs of CNS infiltration existed
- Measurement of Pgrn level in serum using ELISA in 40 healthy persons and in the 40 patients at the time of diagnosis.

Inclusion criteria:

Patients aged and older than 16 years and patients with newly diagnosed AML.

Exclusion criteria:

Patients below age of 16 years, patients with relapsed AML, patients with other malignancy, patients with rheumatological diseases, and patients with neurodegenerative diseases.

Timing of samples:

Sampling for Measurement of serum Pgrn has been extracted after diagnosis of the case and before treatment. The patients have been followed up for a maximum period of 13 months and survival and remission status were assessed at day 28 and at the end of the study.

Methods:

Measuring Pgrn in serum by The Cloud-Clone Corp.™ (USA) Human Progranulin (PGRN) ELISA Kit which is an enzyme-linked immunosorbent assay

Principle of the Assay

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human Progranulin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Progranulin present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human Progranulin is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Progranulin bound in the initial step. The color development is stopped and the intensity of the color is measured.

Calculation of Results:

Pgrn conc. in each sample was determined by locating the O.D. value on the Y axis then extending a horizontal line to the standard curve and at the point of intersection drawing a vertical line to the X axis then the interpolated value obtained was multiplied by the dilution factor to determine amount of Pgrn in the sample.

Sensitivity: 10 pg/ml

Statistical Analysis:

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 23. The quantitative data were presented as mean, standard deviations and ranges when parametric. Also qualitative variables were presented as number and percentages.

The comparison between groups regarding qualitative data was done by using Chi-square test and/or Fisher exact test only when the expected count in any cell found less than 5.

The comparison between two independent groups regarding quantitative data with parametric distribution was done by using Independent t-test.

The comparison between more than two independent groups regarding quantitative data with parametric distribution was done by using One Way ANOVA.

Spearman correlation coefficients were used to assess the correlation between two quantitative parameters in the same group.

Receiver operating characteristic curve (ROC) was used to assess the best cut off point with its sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and area under curve (AUC). Kaplan-Mayer analysis was used to assess the relation of Pgrn with overall survival and disease free survival by using Log-Rank test.

Overall survival (OS) definition: the length of time from the date of diagnosis to the date of death due to any cause.

Disease free survival (DFS) definition: the length of time from the date of complete remission to the date of relapse. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as follows:

P > 0.05: Non significant (NS). P < 0.05: Significant (S). P < 0.01: Highly significant (HS)

Results

		Control group	Patients group	Test value	P-value	Sig.
		No. = 40	No. = 40			
Age (years)	Mean±SD	43.93 ± 17.45	43.73 ± 17.46	0.051•	0.959	NS
	Range	17 – 75	17 – 75			
Sex	Male	25 (62.5%)	19 (47.5%)	1.818*	0.178	NS
	Female	15 (37.5%)	21 (52.5%)			

•: Independent t-test,, *:Chi-square test

Table 1: Comparison between control & patients regarding demographic data (Age and Sex)

		Patients group (No. = 40)	
		No.	%
Comorbidity	No	31	77.5%
	DM	1	2.5%
	HTN	4	10 %
	Cardiac	1	2.5%
	Hepatic	3	7.5%
Extramedullary disease	Negative	20	50 %
	HSM + LN	20	50 %
	CNS	0	0

Table 2: Baseline clinical data of the patients

		Patients group (No. = 40)	
TLC (x103/µl)	Mean ± SD	39.35 ± 67.96	
	Range	0.80 – 335	
HGB (gm/dl)	Mean ± SD	7.62 ± 1.94	
	Range	4 – 13	
PLT (x103/µl)	Mean ± SD	58.20 ± 51.5	
	Range	4 – 220	
LDH (U/L)	Mean ± SD	924.88 ± 687.39	
	Range	147 – 2890	
BM Blasts (% of ANC)	Mean ± SD	64.30 ± 23.82	
	Range	8 – 99	
		No.	%
FAB subtype	M1-2	24	60.0 %
	M3	5	12.5 %
	M4-5	9	22.5 %
	M6	1	2.5%
	M7	1	2.5%
Cytogenetics *	Low	15	37.5 %
	Intermediate	21	52.5 %
	High risk	4	10.0 %

* Low: 7 patients (17.5%) with t(8;21), 3 patients (7.5%) with inv(16), 5 patients (12.5%) with t(15;17), Intermediate: 21 patients (52.5%) with normal cytogenetics, High: one patient (2.5%) with t(9;22), and 3 (7.5%) patients with complex cytogenetic abnormalities

Table 3: Baseline laboratory data of the patients

		Patients group (No. = 40)	
		No.	%
Chemotherapy	3+7	28	71.8%
	Pethema	4	10.3%
	Palliative**	7	17.9%

** 2+5 in 2 patients (5.1%), Ara-C in 4 patients (10.2%), Etoposide in 1 patient (2.5%). One patient did not receive ttt as she died after diagnosis (DIC)

Table 4: Lines of chemotherapy received

		Patients group (No. = 40)	
		No.	%
Survival status at d 28	Alive	29/40	72.5 %
	Dead	11/40	27.5 %
Remission/ Survival status of patients at d 28	Remitted & alive	22/40	55 %
	Resistant or died	18/40	45 %
Remission status of living patients at d 28	Remitted and alive	22/29	75 %
	Resistant and alive	7/29	25 %

Table 5: Outcome of patients at day 28

		Remitted patients group (No. = 22)	
		No.	%
Follow up of d28 remitted patients	Still remitted	19	87 %
	Relapsed	3	13 %

Table 6: Outcome at the end of study

		Patients group (No. = 40)	
		No.	%
Cause of death	Relapse/resistance	9	45 %
	Sepsis	10	50 %
	DIC	1	5%

Table 7: Causes of deaths in patients

		Control group	Patients group	Test value•	P-value	Sig.
		No. = 40	No. = 40			
PGRN at diagnosis	Mean±SD	155 ± 63	346 ± 64	-13.257	0.001	HS
	Range	80 – 370	215 – 545			

•: Independent t-test

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)

Table 8: Comparison between control & patients PGRN

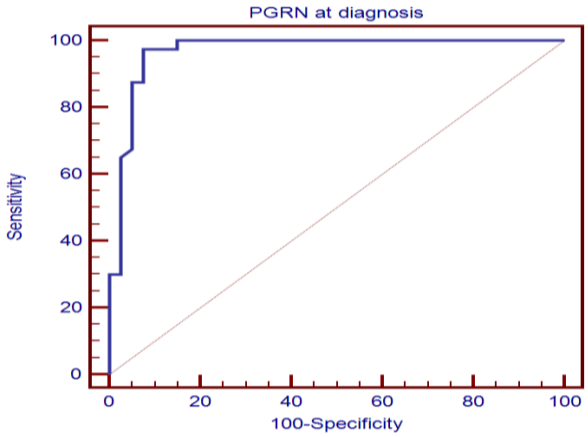

Figure1: ROC curve between (Control & patients) group regarding PGRN at diagnosis for diagnosis of AML.

		PGRN at diagnosis		Test value	P-value	Sig.
		Mean ± SD	Range			
Sex	Male	325.16 ± 60.77	215 – 450	-2.028•	0.050	S
	Female	365.00 ± 63.15	247 – 545			
Type	M1-2	337.92 ± 73.09	215 – 545	0.484••	0.747	NS
	M3	338.00 ± 56.32	292 – 425			
	M4-5	362.33 ± 47.45	300 – 440			
	M6	382.00 ± 0.00	382 – 382			
	M7	400.00 ± 0.00	400 – 400			
Comorbidity	No	344 ± 61.44	245 – 545	0.622••	0.650	NS
	DM	390 ± 0.00	390 – 390			
	HTN	378 ± 79.24	265 – 440			
	Hepatic	306 ± 98.02	215 – 410			
	Cardiac	345 ± 0.00	345 – 345			
Extramedullary disease	Negative	327.60 ± 59.19	215 – 440	-1.870•	0.069	NS
	HSM. LN	364.55 ± 65.64	265 – 545			
Cytogenetics	Low risk	328.40 ± 50.24	245 – 425	1.816••	0.177	NS
	Intermediate	349.38 ± 72.83	215 – 545			
	High risk	395.00 ± 45.28	345 – 450			
Cause of death	Relapse/resistant	347.78 ± 75.05	215 – 450	0.784••	0.472	NS
	Sepsis	349.2 ± 41.21	297 – 415			
	DIC	425 ± 0	425 – 425			

•: Independent t-test••: One Way ANOVA test.

Table 10: Correlation of PGRN with categorical variables

	r (PGRN at diagnosis)	P-value
Age	0.274	0.087
TLC	0.063	0.698
HGB	-0.012	0.942
PLT	-0.033	0.838
LDH	-0.185	0.253
BM Blasts	0.140	0.389

Table 11: Correlation of PGRN with continuous variables

OS (days)		95% Confidence Interval		OS % at end of study
Median	SE	Lower Bound	Upper Bound	
224	60	105	342	44.5 %

Table 12: Overall Survival for whole patients group

DFS (days)		95% Confidence Interval		DFS at end of study
Median	SE	Lower Bound	Upper Bound	
167	26	114	219	45.1 %

Table 13: DFS for whole patients group

PGRN status	Total N	Median	SE	95% CI		Log rank test			OS at end of study
				Lower	Upper	x^2	P-value	Sig.	
< 346	19	259	39	181	338	2.888	0.089	NS	61.6 %
> 346	21	157	33	91	222				23.8 %
Overall	40	214	28	159	269				44.5 %

Table 14: OS for patients with Pgrn < 346 pg/ml and patients with Pgrn > 346 pg/ml

PGRN status	Total No.	Median	SE	95% CI		Log rank test			DFS at end of study
				Lower	Upper	x^2	P-value	Sig.	
< 346	19	219	37	145	293	3.702	0.054	NS	63.2 %
> 346	21	116	33	51	182				27.6 %
Overall	40	167	26	114	219				45.1 %

Table 15: DFS for patients with Pgrn < 346 pg/ml and patients with Pgrn > 346 pg/ml

Discussion

AML is characterized by clonal expansion of undifferentiated myeloid precursors, resulting in impaired hematopoiesis and bone marrow failure. Although many patients with AML have a response to induction chemotherapy, refractory disease is common, and relapse represents the major cause of treatment failure (7).

The growth factor Pgrn has significant biological effects in different types of cancer. This protein is a regulator of tumorigenesis because it stimulates cell proliferation, migration, invasion, angiogenesis, malignant transformation, resistance to anticancer drugs, and immune evasion (1).

Elevated Pgrn levels have been associated with a wide range of different human malignancies such as carcinomas of the breast, ovary, liver, kidney, prostate and the brain. Furthermore, high Pgrn expression levels as detected in the tumor itself or in the peripheral blood have been linked to an aggressive phenotype and poor prognosis in breast cancer, glioblastoma and ovarian cancer (8).

However, data on the role of Pgrn in hematological malignancies are limited. In patients with CLL, high Pgrn plasma levels were strongly associated with adverse risk factors including unmutated IGHV status, expression of CD38 and ZAP-70, and poor risk cytogenetics. Pgrn was prognostic for OS suggesting that Pgrn is a robust and independent prognostic marker in CLL that can be easily measured by ELISA (5). In MM it has been demonstrated in vitro to promote cell survival and confer resistance to dexamethasone treatment (9). In patients with malignant lymphoma, serum Pgrn was significantly higher than normal controls (6).

In line with data from studies in normal individuals (10) and patients with breast and ovarian cancer (8), we found that Pgrn can be easily and reliably measured in the peripheral blood employing a commercially available enzyme-linked immune-sorbent assay. Göbel et al. (5) compared Pgrn mRNA concentrations in immune-magnetically purified CLL cells with Pgrn protein plasma levels in the same patients and observed a highly significant correlation. Furthermore, cell culture studies using purified CLL cells revealed a time dependent secretion of Pgrn into the culture supernatant providing circumstantial evidence that Pgrn concentrations measured in the plasma indeed reflect the amount of Pgrn production in the leukemic cells derived from individual patients.

In our study, Pgrn levels in healthy participants were in the range from 80 to 370 pg/ml with the mean value of 155 pg/ml while in the patients the range were from 215 to 545 with the mean value of 346 pg/ml indicating a highly significant difference between control (healthy persons) group and patients group. This is to some extent similar to what has been reported by Yamamoto et al. (6) who examined the concentration of Pgrn in plasma from 100 normal individuals and 254 malignant lymphoma patients by ELISA and found significantly higher pgrn in patients than the control group. Also, Göbel et al. (5) examined the concentration of Pgrn in plasma from 31 normal individuals and 131 CLL patients by

ELISA and found that CLL patients exhibited significantly elevated pgrn levels as compared to controls with no apparent differences for age and sex.

In our study, Performing ROC curve (receiver operating characteristic curve) between Control & patients groups regarding progranulin at diagnosis for diagnosis of AML, Pgrn (cut-off > 235 pg/ml) was sensitive (97.5%) and specific (92.5%) making it a potential beneficial diagnostic marker for AML.

In our study, the correlations between Pgrn level and patient age, Hemoglobin concentration, TLC, platelet count, LDH and BM blasts percentage at the time of diagnosis were statistically insignificant. This is not in line with Göbel et al. (5) who found a clear positive association in patients with CLL between increasing leukemic cells and Pgrn plasma levels. This discrepancy may be explained by the chronic nature of CLL in contrast to the acute rising of blast count in AML and to the limited number of patients in our study. However; in our study, Pgrn levels were higher in patients with high tumour burden (patients suffering from hepatosplenomegaly and/or lymphadenopathy) when compared to the patients with low tumour burden (patients without extramedullary disease) with a tendency towards significance.

In our study, Pgrn levels were significantly higher in female patients when compared to male patients. This is in contrary to Göbel et al. (5) who did not found a significant difference between male and female patients with CLL. This can be explained by the relatively higher frequency of female patients in our study (52.5%) in comparison to the number of female patients in Göbel et al. (5) study (45 %). However, Nicholson et al. (11) also, observed higher plasma pgrn level in females than males.

In our study, Pgrn levels in patients with low-risk cytogenetics were the lowest and in patients with high-risk cytogenetics were the highest but this was statistically insignificant. This is not completely in line to Göbel et al. (5) who observed strong association between high Pgrn plasma levels and high risk cytogenetics in patients with CLL. This can be explained by the low number patients with highrisk cytogenetics in our study (10 %) in comparison to the number of patients with high-risk cytogenetics in Göbel et al. (5) study (33 %).

In our study, we evaluated the prognostic value of Pgrn in our AML patients using the mean Pgrn level of 346 pg/ml to define two patient subgroups with low- versus high risk disease. After a mean duration of follow up of 140 days (7-392) for our patients, Kaplan-Meier analyses revealed shorter OS and DFS times in the Pgrn high versus low patient subgroups but this was statistically insignificant in case of OS. However, there was a tendency towards significance in case of DFS. This is not completely in line to Göbel et al. (5) who reported statistically highly significant differences in terms of OS between the two groups in patients with CLL and Yamamoto et al. (6) who also observed a strong association between Pgrn level and OS in patients with DLBCL. This inconsistency may be due to the limited number of patients in our study and to the more aggressive nature of the disease in our study.

Conclusion

1. PGRN level is high in AML and has high sensitivity and specificity as a diagnostic marker for AML.

2. PGRN level may correlate with tumor burden in AML.

3. AML patients with high Pgrn tend to have inferior DFS and can be, therefore, used as a prognostic marker for DFS in AML.

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