International Journal of Medical Toxicology & Legal Medicine **Volume 27, No. 4S, 2024**
e-ISSN: 0974-4614
p-ISSN: 0972-0448
https://doi.org/10.47059/ijmtlm/V27I4S/037

Diagnostic and Prognostic Value of Serum Levels of Progranulin in Egyptian Multiple Myeloma Patients

Manal ElhuseinyAbufarha[1], Haytham Mohammad Mostafa[2*], Ibrahim Ahmed Abdalgwad[3]

[1,2,3]Clinical Hematology Unit, Internal Medicine Department, Faculty of Medicine, Cairo University, Egypt.
Email:Haytham3mostafa@gmail.com
[*]Corresponding Author

Received: 25.10.2024 Revised: 05.12.2024 Accepted: 17.12.2024

ABSTRACT
Background: Cancer cells have defects in regulatory mechanisms that usually control cell proliferation and homeostasis. They 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 Multiple myeloma and correlating it with diagnosis and prognosis.
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 unit during the period from July 2021 to Jan 2022.
Results: We evaluated the diagnostic value of progranulin in MM patients using the Progranulin level of 20 pg/ml that was sensitive (97.5%) and specific (55%) to differentiate MM patients from healthy controls making it a potential beneficial diagnostic marker for MM. Also We evaluated the prognostic value of progranulin in MM patients using the Progranulin level of 34 pg/ml to define two patient subgroups with low- versus high level correlated respectively with good response versus partial response to treatment after 12 weeks of chemotherapy
Conclusion: Serum pgrn level is significantly higher in MM patients than in healthy individuals making pgrn a diagnostic biomarker for MM. Also, Serum pgrn level is significantly higher in MM patients who showed resistance to chemotherapy VCD making progranulin a beneficial prognostic marker in the choice of treatment protocol.

Keywords: Progranulin – Multiple Myeloma

INTRODUCTION

Multiple Myeloma (MM) is a plasma cell dyscrasia characterized by multifocal proliferation of terminally differentiated, heavy chain class switched, immunoglobulin secreting plasma cells, usually with M-protein and evidence of organ damage related to the plasma cell neoplasm (Pulte et al., 2020). Diagnosis requires the presence of one or more myeloma defining events (MDE) plus evidence of either 10% or more clonal plasma cells on bone marrow (BM) study or a biopsy proven plasmacytoma. MDE consist of established CRAB (hypercalcemia, renal failure, anemia, or lytic bone lesions) features as well as three specific biomarkers: clonal BM plasma cells ≥60%, serum free light chain (FLC) ratio ≥100 (provided involved FLC level is ≥100 mg/L), and more than one focal lesion on MRI (Rajkumar, 2020). Cancer cells have defects in regulatory mechanisms that control cell proliferation and homeostasis. Tumorigenesis requires series of events that include constant cell proliferation, promotion of metastasis and invasion, stimulation of angiogenesis, evasion of tumor suppressor factors & avoidance of cell death pathways. These events may be regulated by growth factors (GF) produced by normal or malignant cells (Arechavaleta et al., 2017). The GF Progranulin (Pgrn) is implicated in multiple biological and pathological processes, such as cell growth, tumorigenesis, embryogenesis, wound healing, inflammation, immunity, infection, diabetes. This protein is a regulator of tumorigenesis as it stimulates cell proliferation, migration, invasion, angiogenesis, malignant transformation, resistance to anticancer drugs, and immune evasion (Neill et al., 2016). Elevated Pgrn level was observed in various types of malignancies such as breast, ovary, liver, kidney, prostate and hematological malignancy. In breast cancer Pgrn has been implicated in

tumorigenesis and resistance to anti-estrogen therapies for estrogen receptor positive breast cancer. Pathological studies showed that Pgrn is expressed in invasive ductal carcinoma, but not in normal mammary epithelial tissue or benign lesions (Elkholy et al., 2019). 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) (Yamamoto et al., 2017). In patients with AML, Pgrn is over-expressed and patients with high-plasmapgrn levels have poor response to chemotherapy. Pgrn was demonstrated in vitro to promote cell survival in MM and confer resistance to dexamethasone (Qin et al., 2020).

Aim of the Work

Aim of this study is to investigate levels of Pgrn in the serum of Egyptian adult patients with MM and its importance as a potential biomarker in diagnosis of MM. Next, to correlate that to prognosis.

Subjects and Methods

This study was conducted on 80 subjects (40 patients and 40 age and sex matched healthy persons) of age between 18 and 65 years and both sexes. Patients were attendants to Clinical Hematology and Oncology Hospital. They were investigated during the period from 1/7/2021 to 1/1/2022. They were divided as follows:
Patients group: 40 Patients with newly diagnosed MM.
Control group: 40 age and sex matched apparently healthy individuals.

Inclusion criteria

Patients aged between 18 and 65 years (Transplant eligible).
Patients with newly diagnosed MM; only de novo cases who will receive VCD regimen (Valcade 1.3 mg/m2, Cyclophosphamide 300 mg/m2, Dexamethazone 40 mg weekly for 12 weeks).
Patients who are Transplant eligible (Good performance status, Adequate organ function, No significant comorbidities)

Exclusion criteria

Patients with other malignancies as cancer breast, kidney and ovary and other hematological malignancies as AML, ALL, CLL and Lymphomas.
Patients with neurodegenerative or rheumatological diseases.
Patients with Refractory\Relapsed MM.

Ethical consideration

The study takes into consideration the basic principle of biomedical ethics for participant patients. Free and voluntary written informed consent was obtained from patients. Guardians were informed about their absolute rights to be involved or, to withdraw at any time of the study. Personal privacy and confidentiality of the corrected data was secured.

Methods

All subjects were submitted to the following

Full history taking and Clinical examination
Routine laboratory: Complete blood count, Kidney & Liver functions tests.
Disease-specific labs: BM aspiration (stained smears by Leishman/ Giemsa) & biopsy (stained by H&E), immune-histochemistry (CD138) and serum/urine protein electrophoresis and serum/urine immunofixation at the time of diagnosis and at the time of assessment of response to treatment (after 4 courses of VCD).
Measurement of progranulin level in serum using ELISA in 40 healthy persons and in the 40 patients at the time of diagnosis.

Measurement of serum progranulin

Serum Pgrn was analyzed by ELISA, with a complete set of ELISA reader model SLT Spectra 216687, using Progranulin ELISA Kit (Cat.No E1755Hu), standard curve range from10 ng/ml to 700 ng/ml, sensitivity is 5.12 ng/ml, supplied by Bioassay Technology Laboratory, China.

Preparation of sample

Thawed samples should be brought to room temperature just prior to the assay and avoid repeated freeze/ thaw cycle, which may cause erroneous results. Haemolyzed or lipemic samples were avoided.

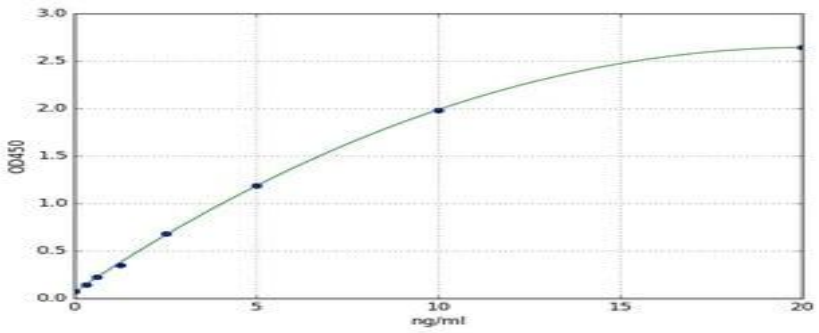
Principle of the technique

This assay employs the quantitative sandwich enzyme-linked immunosorbent assay. A monoclonal antibody specific for Pgrn has been pre-coated onto a micro plate. Standards and samples are pipetted into the wells and

any Pgrn present is bound to antibodies coated on the well. After washing away any unbound substances, a Biotinylated human Pgrn Antibody was added and bound to human Pgrn. Then Streptavidin-Horseradish Peroxidase (HRP) was added and bound to the Biotinylated Pgrn antibody. After incubation unbound Streptavidin-HRP was washed away during a washing step. Substrate solution was then added and color developed in proportion to the amount of human Pgrn. The reaction terminated by addition of acidic stop solution and absorbance was measured at 450 nm. A standard curve was constructed by plotting absorbance values against concentrations of standard.

Calculation of results

The standard curve was generated by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and a best fit curve is drawn through the points on the graph. These calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis.

Figure (1): Standard curve for Progranulin	

Statistical analysis

Data were collected, revised, coded and entered to Statistical package for social science (IBM-SPSS), version 22.

1-Descriptive statistics

The quantitative data were presented as mean, standard deviations (SD) and ranges when their distributions were found parametric. Data were presented as median with interquartile range (IQR) when their distributions were found non-parametric. Also qualitative variables were presented as numbers and percentages.

2-Analytical statistics:

Chi-Square test ($\chi 2$) was used to compare between 2 study groups with quantitative variables (examine relationship between 2 qualitative variables).

Independent t-Test was used to compare between two study groups with quantitative and parametric distribution.

The interquartile range (IQR): is a measure of statistical dispersion, being equal to the difference between 75th and 25th percentiles, or between upper and lower quartiles.

One Way ANOVA: was used to compare between more than two independent groups regarding quantitative data with parametric distribution.

Mann Whitney Test (U test): was used to compare two study groups with quantitative but non-parametric variables.

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

The probability of being by chance (P- value): It was evaluated as follows: $P \geq 0.05$: Non significant (NS). $P < 0.05$: Significant (S). $P < 0.001$: Highly significant (HS).

Receiver Operating Characteristic curve (ROC-curve): used to illustrate the diagnostic properties of a test on a numerical scale and to assess the best cut off point with its sensitivity, specificity, positive predictive value, negative predictive value and area under curve.

Sensitivity: Probability that the test results will be positive when the disease is present (true positive rate, expressed as a percentage).

Specificity: Probability that the test results will be negative when the disease is present (true negative rate, expressed as a percentage).

PPV: Positive Predictive value (probability that the disease is present when the test is positive).

NPV: Negative Predictive value (probability that the disease is present when the test is negative).

Accuracy: the ratio of the true positive and true negative on all patients.

Results

In this study 40 patients with MM were selected and compared to 40 control group.

Table 1: Comparison between control & patients regarding Age

Age		Control group	Patients group	Test value	P-value	Sig.
		No. = 40	No. = 40			
	Mean ± SD	55.85 ± 7.39	57.93 ± 10.75	-1.006•	0.318	NS
	Range	47 – 86	40 – 86			

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01
•: Independent t-test

This table shows no statistically significant difference between studied groups as regard age. Mean age of control group was 55 and mean age of patient group was 57 years old.

Figure (2): Comparison between studied groups as regard age.

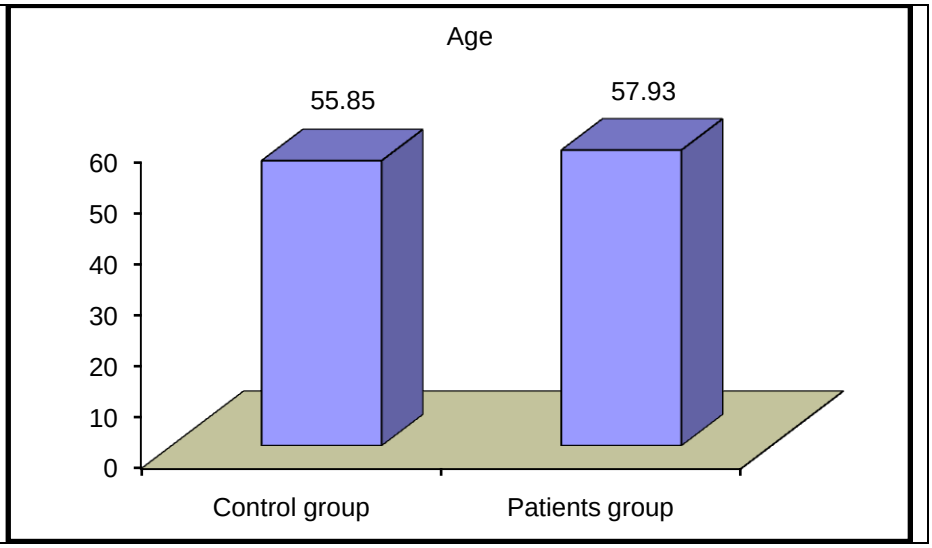


Table 2: Comparison between control & patients regarding Sex

Sex		Control group	Patients group	Test value	P-value	Sig.
		No. = 40	No. = 40			
	Female	19 (47.5%)	21 (52.5%)	0.200*	0.655	NS
	Male	21 (52.5%)	19 (47.5%)			

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01
*:Chi-square test;

This table shows no statistically significant difference between studied groups as regard sex. There were 21 males (52.5%) & 19 females (47.5%) in control gp while there were 19 males (47.5%) & 21 females (52.5%) in patients gp.

Figure (3): Comparison between studied groups as regard sex

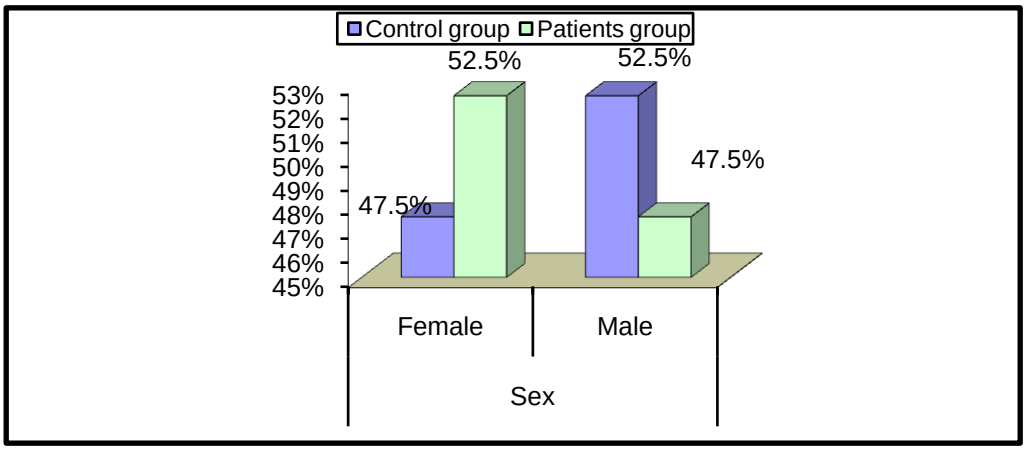


Table 3: Basline laboratory & radiological data of patients

		No. = 40
Hb	Mean ± SD	8.40 ± 1.71
	Range	6 – 11
Anemia (Hb<12)	Negative	0 (0.0%)
	Positive	40 (100.0%)
PLT	Mean ± SD	287.00 ± 95.33
	Range	110 – 420
Thromboctopenia (PLT<150)	Negative	36 (90.0%)
	Positive	4 (10.0%)
TLC	Mean ± SD	6.88 ± 1.99
	Range	4 – 10
Creat	Mean ± SD	1.95 ± 0.72
	Range	0.5 – 3
AKI (Creat>1.2)	Negative	8 (20.0%)
	Positive	32 (80.0%)
Ca	Mean ± SD	9.84 ± 1.17
	Range	7 – 11.3
Hypercalcemia (Ca>10.1)	Negative	21 (52.5%)
	Positive	19 (47.5%)
Albumin	Mean ± SD	2.80 ± 0.58
	Range	2 – 4
M-ptn	Mean ± SD	3.05 ± 0.59
	Range	2 – 4
Plasma%	Median (IQR)	41 (25 – 66)
	Range	10 – 90
Lytic BL	Negative	8 (20.0%)
	Positive	32 (80.0%)

This table shows that 100% of patients have anemia, 10% have thrombocytopenia, 80% have acute kidney injury (AKI), and 47.5% of patients have hypercalcemia while 80% of patients have Lytic bony lesions (BL)

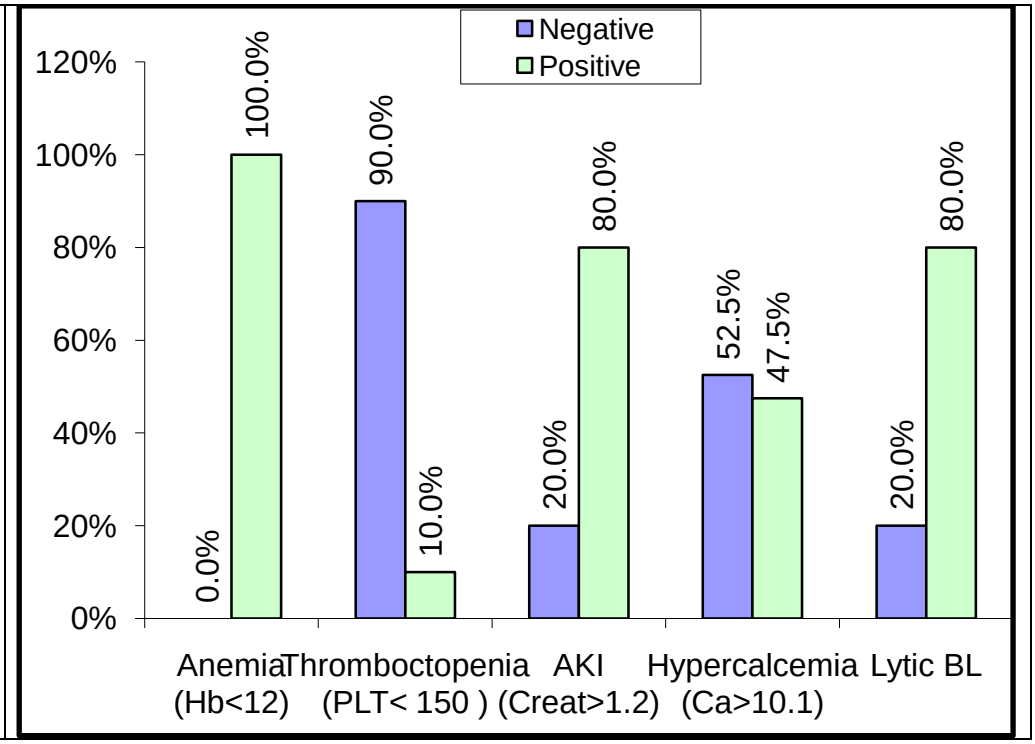


Figure (4): Basline laboratory and radiological data of the patients.

Table 4: Comparison between control & patients PGRN

		Control group	Patients group	Test value	P-value	Sig.
		No. = 40	No. = 40			
serum Pgrn	Mean ± SD	19.53 ± 5.88	30.00 ± 5.86	-7.977•	0.000	HS
	Range	10 – 30	20 – 40			

Significant (S); P-value< 0.01: highly significant (HS)
•: Independent t-test

This table shows mean Pgrn level in sera of Healthy persons and Patients with MM and This table shows statistically significant difference (p-value < 0.05) between studied groups as regardPgrn. In control group the range was from 10 – 30 ng/ml with the mean value of 19.5 ng/ml while in the patients the range was from 20 – 40 ng/ml with the mean value of 30 ng/ml.

Figure (5): Comparison between control & patients PGRN

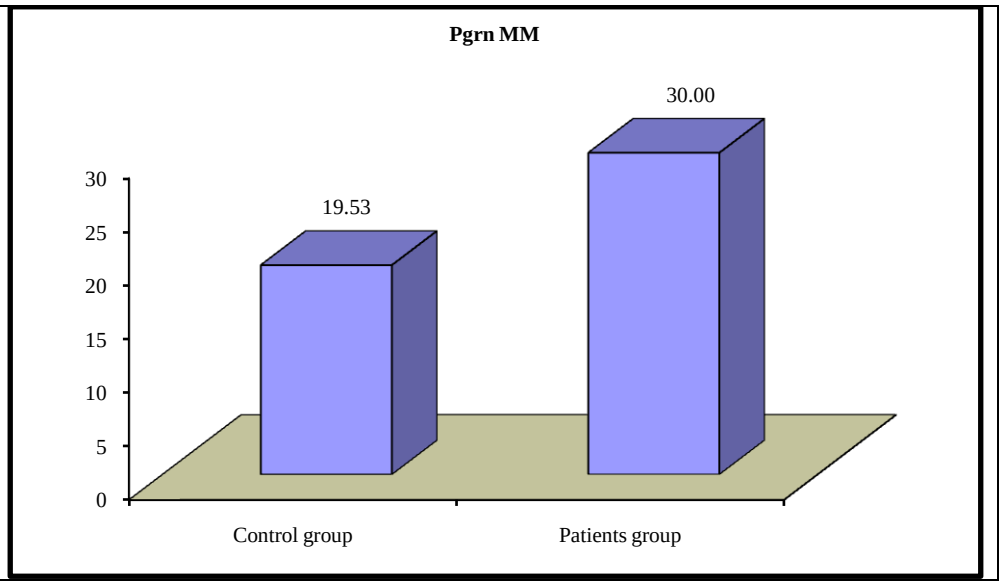


Table 5: Diagnostic performance of PGRN to predict patients with multiple myloma

Parameter	AUC	Cut of Point	Sensitivity	Specificity	PPV	NPV
Pgrn	0.886	>20	97.5	55.0	68.4	95.7

PPV: positive predictive value. NPV: negative predictive value.
AUC: Area under curve

Using ROC curve, it was shown that Pgrn can be used to discriminate between patients & control at a cutoff level of > 20 ng/ml, with 97.5% sensitivity, 55% specificity, 68.4% PPV and 95.7% NPV

Figure (6): ROC curve between control & patients as regardPgrn.

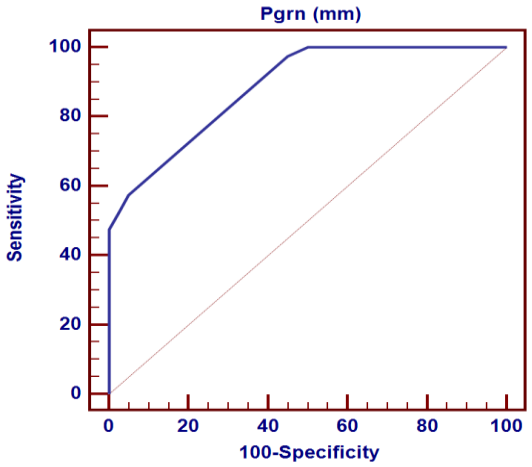


Table 6: Correlation between PGRN and laboratory data of patients

	Pgrn MM	
	r	P-value
Age	-0.114	0.485
Hb	0.251	0.119
PLT	-0.215	0.183
Creat	0.533*	0.000
Ca	-0.050	0.760
Albumin	-0.383*	0.015
M-ptn	0.376*	0.017
Plasma%	0.100	0.541

***Spearman correlation coefficients**

In Patients group there were:
High statistically significant (p-value < 0.000) Positive correlation (r = 0.533) between Pgrn and creatinine.
Statistically significant (p-value = 0.015) Negative correlation (r = - 0.383) between Pgrn and Albumin.
Statistically significant (p-value = 0.017) Positive correlation (r = 0.376) between Pgrn and M protein.

Figure (7): Positive correlation between Pgrn and serum creatinine in patients group

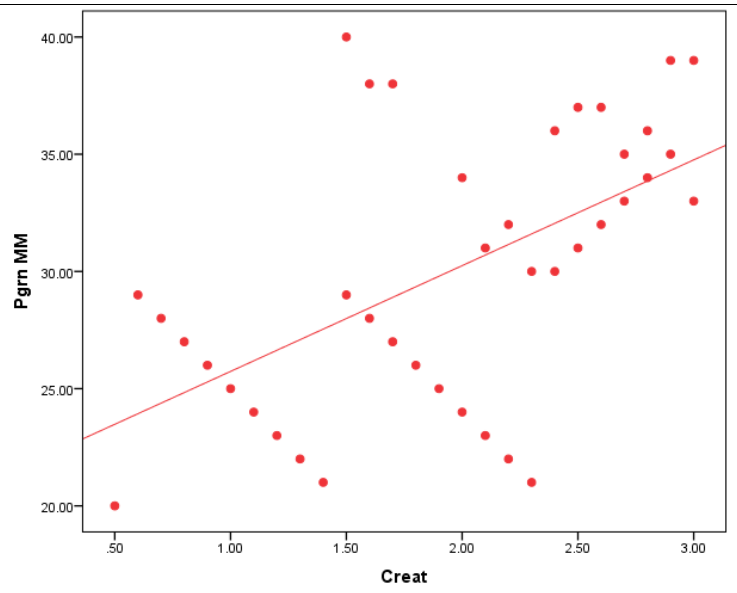


Figure (8): Negative correlation between Pgrn and Albumin in patients group.

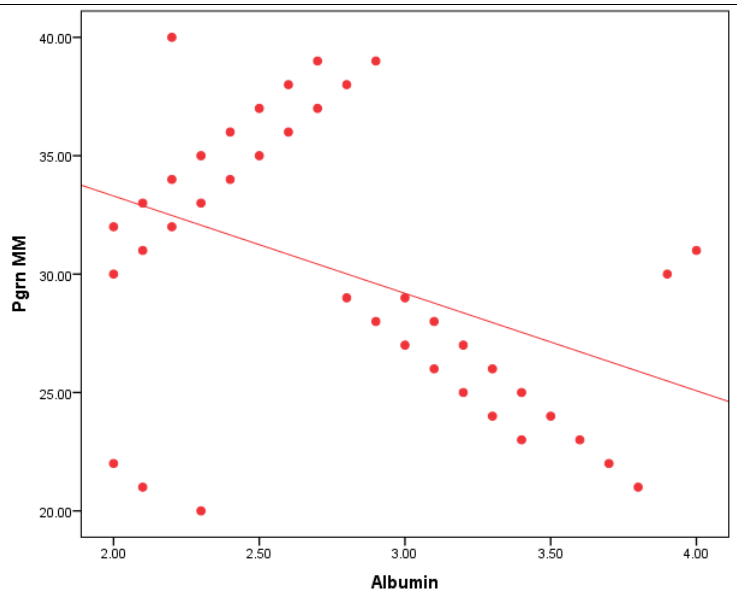


Figure (9):Positive correlation between Pgrn and M protein in patients group.

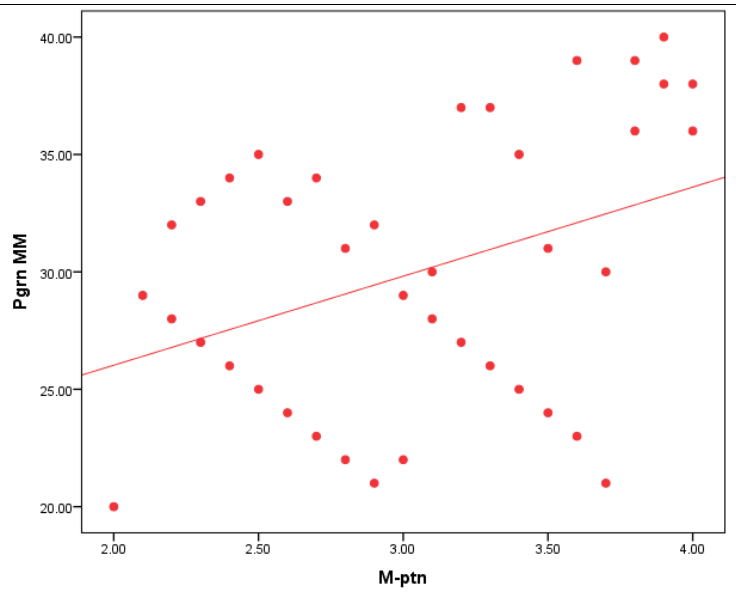
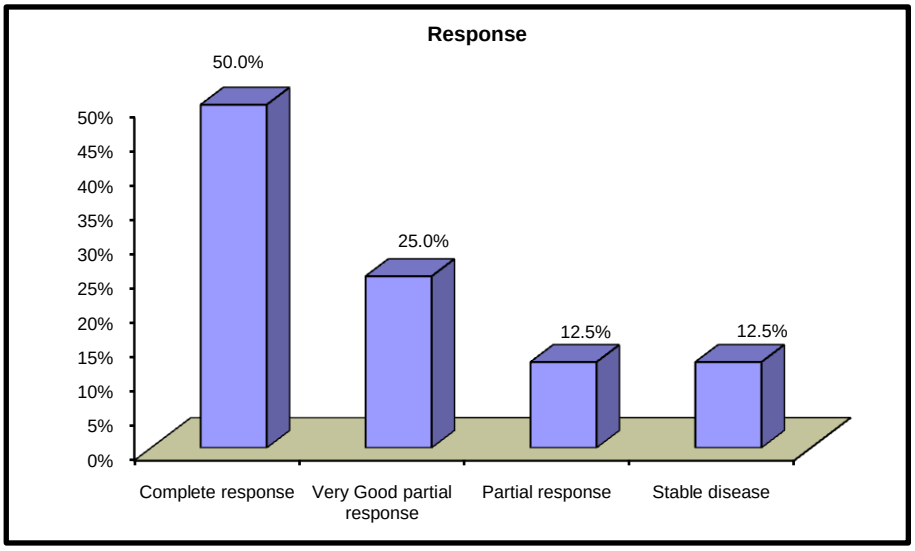


Table 7: Outcome of patients after therapy

Response	No.	%
Complete response (CR)	20	50.0%
Very Good partial response (VGPR)	10	25.0%
Partial response (PR)	5	12.5%
Stable disease (SD)	5	12.5%

This table shows the number and percentage of patients that achieved CR, VGPR, PR and stable disease after end of chemotherapy (4 groups)

Figure (10): Outcome of patients after therapy
After end of chemotherapy; there are 4 groups where: the percentage of patients that achieved CR is 50%, the percentage of patients that achieved VGPR is 25% the percentage of patients that achieved PR is 12.5%, then percentage of patients that remained stable disease was 12.5



Prognostic performance of PGRN

Table 8: Comparison between the 4 groups of patients (acc.to response after VCD) regarding Pgrn

		CR	VGPR	PR	SD	Test value•	P-value	Sig.
		No. = 20	No. = 10	No. = 5	No. = 5			
Pgrn	Mean±SD	25 ± 2.99	32.5 ± 1.58	36.2 ± 0.84	38.8 ± 0.84	69.171•	<0.001	HS
	Range	20 – 30	30 – 35	35 – 37	38 - 40			

• One Way ANOVA test m P-value< 0.01: highly significant (HS)

Post Hoc analysis by LSD	Complete response Vs Very Good partial response	Complete response Vs partial response	Complete response Vs Stable disease	Very Good partial response Vs partial response	Very Good partial response Vs Stable disease	Partial response Vs Stable disease
Pgrn MM	0.000	0.000	0.000	0.007	0.000	0.088

Figure (11): Comparison between the 4 groups of patients (acc.to response to VCD) regarding Pgrn

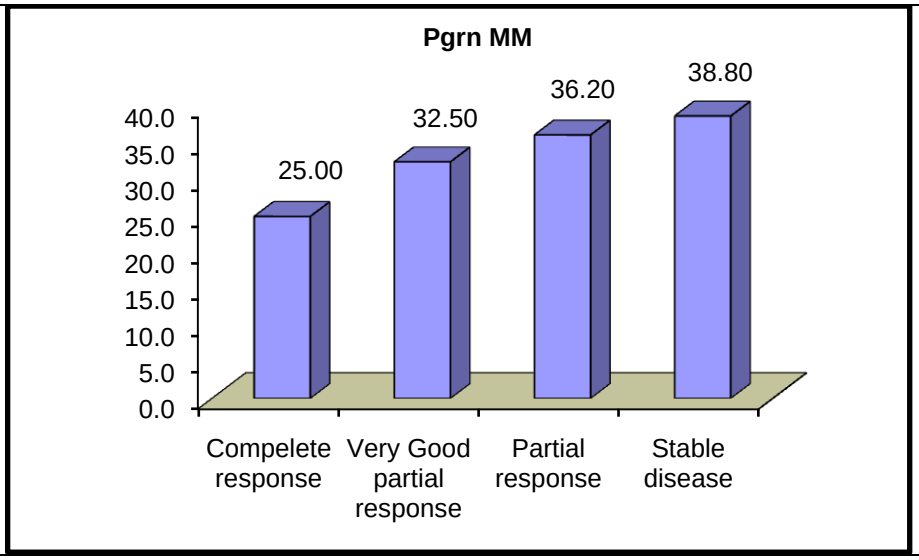


Table 9: Comparison between 2 groups of patients

Response		Pgrn MM		Test value	P-value	Sig.
		Mean ± SD	Range			
	CR+ VGPR	27.5 ± 4.42	20 – 35	6.950	<0.001	HS
	PR+Stable disease	37.5 ± 1.58	35 – 40			

when we make the patients who achieved CR and patients who achieved VGPR as one group (as those are the patients who will go for ASCT) and the patients who achieved PR and those who remained in stable disease as another group and then compared the 2 groups regarding Pgrn, the table shows that Pgrn was significantly higher in the 2nd group

Figure (12): Comparison between 2 groups of patients

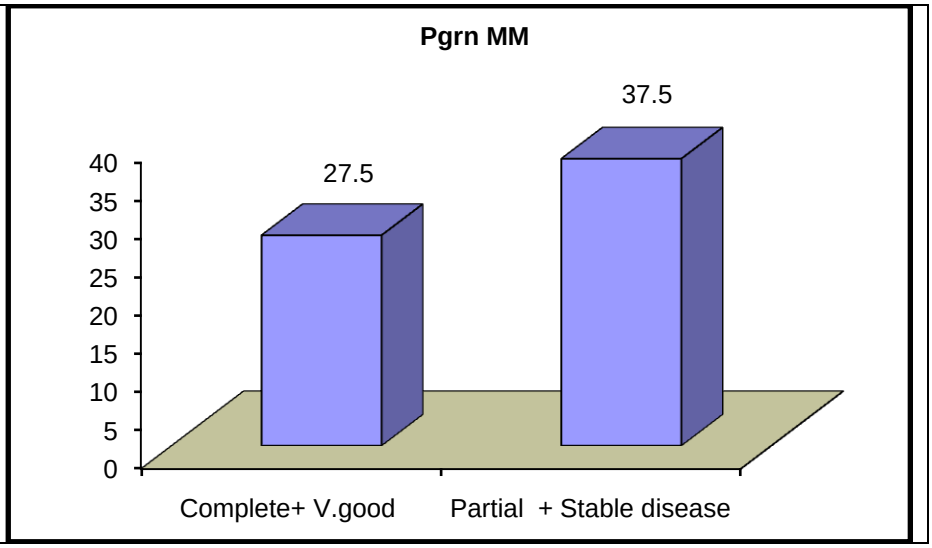
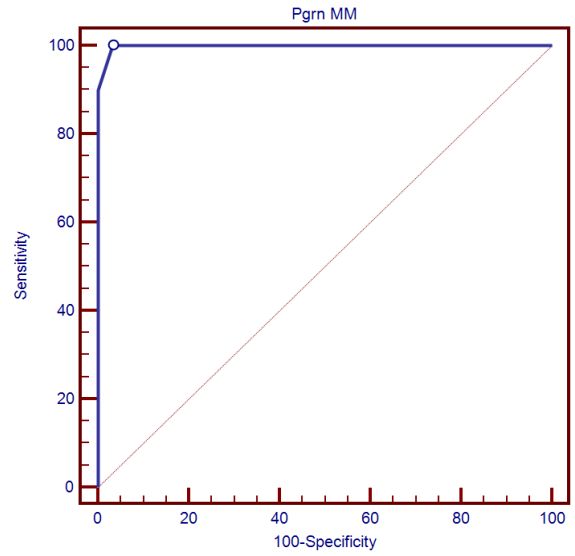


Table 10: Prognostic performance of Pgrn to differentiate patient who will go for ASCT from those who will not

Cut off point	AUC	Sensitivity	Specificity	+PV	-PV
>34	0.998	100.00	96.67	90.9	100.0

Using ROC curve, it was shown that Pgrn can be used to discriminate between patients who will directly go for ASCT and those who will not at a cutoff level of > 34 ng/ml, with 100 % sensitivity, 96% specificity, 90% PPV and 100% NPV

Figure (13): Receiver operating characteristic curve (ROC) for Pgrn to differentiate patients with complete and very good response from those with partial or stable response



DISCUSSION

Diagnosis of MM requires the presence of one or more myeloma defining events (MDE) in addition to evidence of either 10% or more clonal plasma cells on BM examination or a biopsy-proven plasmacytoma. MDE consists of established CRAB (hypercalcemia, renal failure, anemia, or lytic bone lesions) features as well as 3 specific biomarkers: clonal BM plasma cells ≥60%, serum free light chain (FLC) ratio ≥100 (provided involved FLC level is ≥100 mg/L), and more than one focal lesion on MRI (Rajkumar, 2022).

MM patients have diverse symptoms so simple and effective means of testing are beneficial to the patient and the doctor. Non-invasive, cost-effective and convenient new applications are emerging to overcome any limitations and blood-based biomarkers are ideal to meet these needs (Pan et al., 2018).

The GF Pgrnwere observed to be elevated in various human malignancies such as breast, ovary, liver, kidney, prostate, brain cancer and hematological malignancies (Elkholy et al., 2019). 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 (Koo et al., 2012)

Highly significant correlation was observed between Pgrn mRNA concentrations in immune-magnetically purified CLL cells and Pgrn plasma levels in the same patients. Furthermore, cell culture studies using purified CLL cells revealed a time dependent secretion of Pgrn into the culture supernatant providing evidence that Pgrnconcentrations measured in the plasma indeed reflect the amount of Pgrn production in the leukemic cells (Göbel et al. 2013).

The aim of the study is to measure level of Progranulin in the serum of patients with Multiple myeloma and its importance as a potential biomarker in diagnosis of MM. Next, to correlate that with clinical outcome. This study was conducted on 80 subjects; 40 patients with MM (19 male and 21 female patients with mean age around 57 years old) who were attendants to Hematology/Oncology hospital and 40 age and sex matched healthy participants. We found that Pgrn can be easily and reliably measured in the peripheral blood employing a commercially available enzyme-linked immune-sorbent assay (ELISA) and this is in agreement with data from studies in normal individuals (McDade et al., 2012) and patients with breast and ovarian cancer (Koo et al., 2012).

In our study, Progranulin levels in healthy participants were in the range from 10 to 30 pg/ml with the mean value of 19.5 pg/ml while in the MM patients the range were from 20 to 40 with the mean value of 30 pg/ml indicating a highly significant difference between control (healthy persons) and patients. This is close to El-Ghammaz et al. (2020) who examined the concentration of pgrn in plasma from 20 normal individuals and 40 adults ALL patients by ELISA and found that, pgrn mean in healthy control group were 22 ± 6.5 ng/ml and also found significant difference between patients and controls regarding serum Pgrn level. This is in agreement to what has been reported by Yamamoto et al. (2017) 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. (2013) examined the concentration of Progranulin 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. Again, Qin et al. (2020) found highly significant difference between AML patients and control groups as regards pgrn. Also, Elkholy et al. (2019) proved that, serum pgrn was significantly higher in patients with Diffuse Large B-cell lymphoma (DLBCL) compared with control group.

Serum pgrn levels in MM patients (median pgrn levels 30 ng/ml) in our study were comparable with the levels detected in AML patients reported by Qin et al. (2020), who examined the concentration of Pgrn in plasma from 33 newly diagnosed AML patients by ELISA and found its mean was 31.2 ng/mL.

However, this levels are considered low if compared to Pgrn levels in malignant lymphoma (median= 92 ng/ml) (Yamamoto et al. 2017), in breast cancer (median= 55 ng/ml) (Tkaczuk et al. 2020), in prostate cancer (mean= 48.7 ng/ml) (Greither et al. 2018), in ALL (mean= 161 ng/ml) (El-Ghammaz et al. 2020) and in rheumatoid arthritis (mean= 65 ng/ml) (Negm et al. 2018). This may be attributable to high serum M protein in cases of MM, which may mask Pgrn epitope recognized by an ELISA antibody.

Performing ROC curve (receiver operating characteristic curve) between Control & patients' groups regarding progranulin at diagnosis for diagnosis of MM, progranulin (cut-off > 20 pg/ml) was sensitive (97.5%) and specific (55%) making it a potential beneficial diagnostic marker for MM.

Our work showed non-significant correlation between pgrn and age. thisis in agreement with El-Ghammaz et al. (2020) but Nguyen et al. (2020) investigated pgrn as a candidate biomarker for frailty in a cohort study of 358 late middle-aged and older and found that plasma pgrn levels increased with age. Also, Greither et al. (2018) measured pgrn in serum of 142 prostate cancer patients and found that pgrn levels increased with age, as high levels of pgrnwas presented in elder patients (>66 years). This could be explained by wide variety of ages in their subjects.

In our study, the correlations between progranulin level and patient age, Hemoglobin concentration, platelet count, and BM plasma cells percentage at the time of diagnosis were statistically insignificant. These results are in agreement with a study by El-Ghammaz et al. (2020) who examined the concentration of pgrn in plasma from adult ALL patients and found no correlation between serum pgrn and Hemoglobin, platelet count, absolute blast count in peripheral blood, percent of blasts in BM. However, Qin et al. (2020) found a clear positive association in AML patients between total leukocyte count and pgrn plasma levels and highly statistical correlation between Pgrn and BM or peripheral blood blasts. Also, Göbel et al. (2013) found positive association between increasing leukemic cells and pgrn plasma levels in CLL patients. This difference could be attributed to the different disease features in these studies.

In our study, there was highly significant positive correlation between pgrn and serum creatinine. This is in agreement with Albeltagy et al. (2019) who found highly significant positive correlation between serum Pgrn and serum creatinine among 60 Egyptian patients with type 2 diabetes mellitus. Also, they reported that serum pgrn was significantly increased in cases of renal affection. The possible explanation may be that it is a compensatory mechanism to reduce renal deterioration, since pgrn could attenuate inflammation in acute conditions (Yoo et al.,2019). Another explanation for elevated circulating pgrn levels may be due to impaired renal clearance of pgrn. That could be evidenced by Nicoletto et al. (2018), who studied changes in pgrn serum concentration overtime after kidney transplantation on 46 patients who underwent kidney transplantation and found reduction in serum pgrn observed immediately after transplantation, that could be attributed to improvement in kidney function.

Our results revealed positive correlation between pgrn and serum M protein. This is to some extant in line with Berghoff et al. (2016) who studied serum pgrn in 270 patients with various neurological diseases and found statistical significance and positive correlation between pgrn and IgG. Increased pgrn with the elevation of M protein may occur as a compensatory mechanism to diminish nephropathy and amyloidosis caused by M protein, as pgrn plays an important role in the regulation of inflammation, tending to suppress the inflammatory response by reducing pro-inflammatory cytokine production such as IL-4, IL-10, and IL-5 and increasing the production of anti-inflammatory cytokines. Moreover, pgrn suppresses the action of tumor necrosis factor-alpha (Townley et al., 2018).

As regard chemotherapy regimen, all patients received VCD for 12 weeks then subjected for evaluation of the response by BM aspirate and biopsy and serum protein electrophoresis. Half of the patients achieved CR, 25% of patient achieved VGPR, 12.5% achieved PR and 12.5% had a stable disease after end of chemotherapy. These response rates to VCD are different from what reported by Moreau et al. (2016) who reported CR 9%, VGPR 47 %, PR 27 % and Sidana et al. (2022) who reported CR 17 %, VGPR 42 %, PR 41%. Whereas Cifteciler et al. (2020) reported CR+VGPR 30% and PR+SD 70% and Leiba et al. (2014) reported CR 17%, VGPR 23, % PR 43%, SD 17 %.

We found significant difference between pgrn level in the sera of these 4 groups (CR, VGPR, PR, and SD groups) and to evaluate the prognostic value of progranulin in our MM patients, we made the patients into 2 groups: the first group included the patients who achieved CR and the patients who achieved VGPR (as those were the patients who would go for ASCT) and the second group included the patients who achieved PR and

those who remained in stable disease and then compared the 2 groups regarding Pgrn, and we found that the Pgrn was significantly higher in the 2nd group.

Performing ROC curve (receiver operating characteristic curve) between the two patients group regarding progranulin, progranulin (cut-off > 34 pg/ml) was sensitive (100%) and specific (96%) making it a potential beneficial prognostic marker for MM.

This is in agreement with Chen et al. (2018) who found that pgrn levels were significantly higher in MM patients compared to healthy controls and that Pgrn showed good diagnostic accuracy in distinguishing MM patients from controls, and its levels correlated with disease stage, and with Wang et al. (2020) who showed that plasma pgrn were significantly elevated in MM patients compared to controls and Pgrn demonstrated good diagnostic accuracy, particularly in distinguishing MM patients from MGUS patients suggesting that plasma pgrn could be a diagnostic biomarker for MM.

Also, Guo et al. (2019) found that pgrn levels were significantly higher in MM patients compared to healthy controls and Higher pgrn levels were associated with advanced disease stage, poor overall survival, and shorter progression-free survival concluding that pgrn could serve as a potential biomarker for diagnosis and prognosis in MM patients.

Also, Shi et al. (2021) reported that plasma pgrn levels were significantly higher in MM patients compared to healthy controls and Pgrn levels correlated with disease stage and were an independent prognostic factor for overall survival and Li et al. (2020) found that pgrn were significantly higher in MM patients compared to healthy controls, and its levels correlated with clinical parameters such as disease stage, LDH, and BM plasma cell infiltration and Pgrn was an independent prognostic factor for overall survival suggesting that pgrn could be a biomarker for diagnosis and prognosis in MM .

While Zhang et al. (2020) showed that MM patients with higher pgrn levels had poorer treatment response and shorter survival outcomes and Pgrn was an independent prognostic factor for overall survival suggesting that pgrn could be used as a biomarker to assess treatment response and predict prognosis in MM.

The study showed that the mean age of patients was 57 years which agreed with Gupta et al. (2019) who examined 30 newly MM patients and found that the mean age was 59 years. Also, this was in agreement with Aref et al. (2020) who studied 50 Egyptian, newly diagnosed MM patients and reported that the mean age of patients was 56.8 years and Hussain et al. (2019) who studied 99 MM patients and found the mean age was 61.8 years. This shows the relation between MM and old age (Schoenbeck and Wildes, 2020).

AT the time of diagnosis, all patients had anemia (mean hemoglobin concentration was 8.4 g/dl), 80% of patients had acute kidney injury (AKI) (mean creatinine concentration was 1.95 mg%), 47% of patients had hypercalcemia (mean calcium level was 9.84 mg%), and 80% of patients had lytic bony lesions. BM study of the 40 patient revealed presence of plasma cells (median percentage of plasma cells to all nucleated cells was 41%). All patients had a monoclonal band in Serum protein electrophoresis with mean concentration of M-band was 3.05 g%.

Relatively comparable results were reported by Hussain et al. (2019) who studied 99 MM patients and reported that, anemia (Hb <10 gm/dl) was initially found in 51% cases, hypercalcemia in 12% (mean 9.4), mean for serum creatinine was 2.0 mg/dL.

In agreement with our finding, Gogia et al. (2018) and Aref et al. (2020) found that serum creatinine was significantly high in MM patients. Conversely, Kumar et al. (2020) found no significant difference between serum creatinine in patients with MM and healthy individuals.

Serum albumin is a significant prognostic factor of MM that reflects the severity of disease progression (Lida et al., 2019). In our study, there was significant decrease in serum albumin in MM patients (Mean Albumin concentration was 2.8 mg%). This agreed with Aref et al. (2020) who found significant decrease in serum albumin in patients with MM but was different from Hussain et al. (2019) who reported that mean for serum albumin was 3.4 g/dl.

In our study, we found that 47% of patients had hypercalcemia i.e. Calcium more than 10.1mg% (mean calcium was 9.84 mg%). Local osteolytic bone lesions are believed to be the main reason for hypercalcemia in patients with MM. Also, renal affection has an important role. Due to the excessive deposition of immunoglobulin in patients with MM, renal tubular function is often impaired, which leads to increased calcium reabsorption by renal tubules. Subsequently, the ability of the kidney to effectively remove excessive calcium from the circulation is hampered, resulting in elevated serum calcium, which may lead to severe hypercalcemia and renal failure (Bao et al., 2020). In agreement with us, Kumar et al. (2020) found significant increase of serum calcium in MM patients in comparison to controls. However, Aref et al. (2020) could not find any significant difference between MM patients and controls as regards serum calcium. This may be explained by hypo-albuminemia in these patients that may interfere with accurate quantitation of calcium.

BM aspirate of MM patients in our study revealed high percentage of plasma cells (median = 41%). Matsue et al. (2019) studied 196 patients with newly diagnosed symptomatic MM at Jaban and found the median BM plasma cells was 70% and Hussain et al. (2019) also found the median BM plasma cells was 66%. The lower percentage of plasma cells in our study in comparison to other studies may be due to early diagnosis of cases in

our health system.
In our study, all patients had serum M-protein by serum protein electrophoresis (mean was 2.5 g/dl) (secretory MM). The same results reported by Diwan et al. (2018) as all cases had M-protein by serum protein electrophoresis. Also, Gupta et al. (2019) examined 30 newly MM patients and found the mean value of M-protein was 2.6 g/dl.
All these data make the Pgrn a promising new molecular target to develop an effective therapy in malignancies (Yabe et al., 2021).

Summary
This work studied progranulin level in 40 healthy individuals and 40 newly diagnosed MM patients and found it significantly higher in sera of myeloma patients than sera of healthy individuals making serum progranulin beneficial diagnostic marker. Also the work followed the patient after receiving 12 week of VCD then grouped the patients into 2 groups according to response; the first included the patients who achieved complete remission or very good partial remission and the second included the patients who achieved partial response and who remained in stable disease and then compared between the level of progranulin in sera of the 2 groups at the time of diagnosis and found significantly higher levels of progranulin in the sera of the second group making progranulin a beneficial prognostic marker to predict the patients who will be set to auto-stem cell transplantation and the patients who will not.

CONCLUSION

We concluded that
Serum pgrn level is significantly higher in MM patients than in healthy individuals making pgrn a diagnostic biomarker for MM
Serum pgrn level is correlated with some prognostic markers of MM as serum creatinine, and M proteins.
Serum pgrn level is significantly higher in MM patients who showed resistance to chemotheapy VCD making progranulin a beneficial prognostic marker in the choice of treatment protocol.

Recommendations
We recommend further studies using larger number of patients, longer duration and multi-center research to validate the present results and to confirm the significance of Pgrn in MM
We recommend further studies to show the prognostic significance of pgrn in MM treated with other protocols
We recommend to measurePgrn level after stem cell transplantation and to determine if it could be used in the follow-up of MM as a marker of disease relapse.
We recommend to search for pgrn therapeutic significance.

REFERENCES

1. Albeltagy ES, Hammour AE and Albeltagy SA (2019): Potential value of serum Proganulin as a biomarker for the presence and severity of micro vascular complications among Egyptian patients with type 2 DM. Journal of Diabetes & Metabolic Disorders; 18(1): 217-228.
2. Arechavaleta-Velasco F, Perez-Juarez C, Gerton G, et al. (2017): Progranulin and its biological effects in cancer. Med Oncol, 34, 194.
3. Aref S, Al Saeed A, El Menshawy N, et al. (2020): Prognostic relevance of telomere length and telomerase reverse transcriptase variant on MM patients. Journal of Clinical Laboratory Analysis; 34(4): e23133
4. Bao L, Wang Y, Lu M, et al. (2020): Hypercalcemia caused by humoral effects and bone damage indicate poor outcomes in newly diagnosed MM patients. Cancer medicine; 9(23): 8962-8969.
5. Berghoff M, Hochberg A, Schmid A, et al. (2016): Quantification and regulation of the adipokines resistin and progranulin in human cerebrospinal fluid. European Journal of Clinical Investigation; 46(1): 15–26.
6. Chen Y, Wang W, Han Q, et al. (2018): Progranulin as a potential diagnostic biomarker for MM. ExpHematolOncol;7:9.
7. Cifteciler R, Goker H, Demiroglu H, et al. (2020): Evaluation of the survival outcomes of MM patients according to their plasmacytoma presentation at diagnosis. Turk JH, 37.256-262.
8. Diwan A G, Gandhi S A, Krishna K, et al. (2014): Clinical profile of the spectrum of MM in a teaching hospital. Medical Journal of Dr. DY Patil University; 7(2): 185.
9. El-Ghammaz AM, Azzazi MO, Mostafa N, et al. (2020): Prognostic significance of serum progranulin level in de novo adult acute lymphoblastic leukemia patients, Clinical and Experimental Medicine; 20 (2):269-276.
10. Elkholy R, Allam A, Elattar S, Elkady M. (2019): Prognostic significance of progranulin and B7-H4 in patients with diffuse large B-cell lymphoma. Egypt J Haematol;44: 82-90.
11. Göbel M, Eisele L, Möllmann M, et al. (2013): Progranulin is a novel independent predictor of disease progression and overall survival in chronic lymphocytic leukemia. PloS one; 8(8): e72107.

12. Gogia A, Sikka M, Sharma S, et al. (2018): Hemostatic abnormalities in MM patients. Asian Pacific Journal of Cancer Prevention: APJCP;19(1): 127.
13. Greither T, Fischer K, Theil G, et al. (2018): Expression of GP88 (progranulin) in serum of prostate cancer patients is associated with Gleason scores and overall survival. Cancer management and research; 10: 4173.
14. Guo Y, Cui Y, Huang J, et al. (2019): Progranulin as a novel biomarker in diagnosis and prognosis of MM. Cancer Biomark;25(4):351-358.
15. Gupta N, Sharma AP and Sharma AL (2019): Emerging biomarkers in MM: A Review. Clinica Chimica Acta 503: 45-53.
16. Hussain A, Almenfi HF, Almehdewi AM, et al. (2019): Laboratory Features of Newly Diagnosed MM Patients. Cureus; 11(5): e4716.
17. Koo DH, Park CY, Lee ES,et al. (2012): Progranulin as a prognostic biomarker for breast cancer recurrence in patients who had hormone receptor positive tumors: a cohort study. PLoS One; 7(6):e39880.
18. Kumar R, Gupta N, Singh V, et al. (2020): Unveiling molecular associations of polymorphic variants of VDR gene (FokI, BsmI and ApaI) in MM patients of Indian population. The Journal of steroid biochemistry and molecular biology; 199:105588.
19. Leiba M, Kedmi M, Duek A, et al. (2014): VCD versus VTD as induction therapies in newly diagnosed transplant eligible patients with MM: a meta-analysis. BJH, 166, 702-710
20. Li X, Li Y, Yu X, et al. (2020): Progranulin: a potential diagnostic and prognostic biomarker in MM. Ann Hematol;99(7):1665-1673.
21. Matsue k, Kobayashi H, Matsue Y, et al. (2019): Prognostic significance of bone marrow abnormalities in the appendicular skeleton of patients with MM. The American Society of Hematology; 2(9):1032-1039.
22. McDade E, Boeve B F, Burrus T M, et al. (2012): Similar clinical and neuroimaging features in monozygotic twin pair with mutation in progranulin. Neurology; 78: 1245–1249.
23. Moreau P, Hulin C, Macro M, et al. (2016): VTD is superior to VCD prior to intensive therapy in MM: results of the prospective IFM2013-04 trial. BLOOD, V 127, N 21
24. Negm A A, Nassr H M, Fathi N A, et al. (2018): AB0108 Relationship between progranulin and musculoskeletal ultrasound in rheumatoid arthritis:1249-1249.
25. Neill T, Buraschi S, Goyal A, et al. (2016): EphA2 is a functional receptor for the GF progranulin. J Cell Biol; 215 (5): 687–703.
26. Nguyen AD, Malmstrom TK, Niehoff ML, et al. (2020): Serum progranulin levels are associated with frailty in middle-aged individuals. PloS one; 15(9): e0238877.
27. Nicoletto BB, Pedrollo EF, Carpes LS, et al. (2018): Progranulin serum levels in human kidney transplant recipients: a longitudinal study. PloS one; 13(3): e0192959.
28. Pan Y, Chen H, Shen X, et al. (2018):Serum level of long noncoding RNA H19 as a diagnostic biomarker of MM, Clinica Chimica Acta; 480: 199–205.
29. Pulte D, Jansen L, Brenner H. (2020): Changes in long term survival after diagnosis with common hematologic malignancies in the early 21st century. Blood Cancer J. 10, 56.
30. Qin J, Huang S, Qian J, et al. (2020): The prognostic relevance and expression of progranulin in adult patients with acute myeloid leukemia. Medicine; 99(1): e18574.
31. Rajkumar SV and Dispenzieri A (2020): MM and related disorders. In Abeloff's Clinical Oncology (Sixth Edition): 1884-1910.
32. Rajkumar V. (2020): MM: update on diagnosis, risk-stratification and management. Am J Hematol; 95: 548– 567.
33. Rajkumar V. (2022): MM: 2022 update on Diagnosis, Risk-stratification and Management. Am J Hematol August ; 97(8): 1086–1107.
34. Schoenbeck K L and Wildes T M (2020): Updated perspectives on the management of MM in older patients: Focus on lenalidomide. Clinical Interventions in Aging; 15: 619.
35. Shi L, Xu L, Zhao X, et al. (2021): Diagnostic and prognostic significance of plasma progranulin in patients with MM. Int J Lab Hematol;43(3):384-390.
36. Sidana S, Kumar S, Fraser R, et al. (2022): Impact of Induction Therapy with VRD versus VCD on Outcomes in Patients with MM in Partial Response or Better Undergoing Upfront Autologous SCT. Transplantation and Cellular Therapy 28; 83.e1_83.e9
37. Tkaczuk K H, Hawkins D, Yue B, et al. (2020): Association of serum progranulin levels with disease progression, therapy response and survival in patients with metastatic breast cancer. Clinical breast cancer; 20(3): 220-227.
38. Townley RA, Boeve BF and Benarroch EE (2018): Progranulin: Functions and neurologic correlations.Neurology; 90(3): 118-125.
39. Wang J, Sun Q, Yang Y, et al. (2020): Diagnostic significance of plasma progranulin in MM. Int J ClinExpPathol;13(5):977-984.

40. Yabe K, Yamamoto Y, Takemura M, et al. (2021): Progranulin depletion inhibits proliferation via the transforming GF beta/SMAD family member 2 signaling axis in Kasumi-1 cells. Heliyon7, e05849
41. Yamamoto Y, Goto N, Takemura M, et al. (2017): Association between increased serum GP88 (progranulin) concentrations and prognosis in patients with malignant lymphomas. Clinica Chimica Acta 473: 139-146.
42. Yoo W, Lee J, Noh K H, et al. (2019): Progranulin attenuates liver fibrosis by downregulating the inflammatory response. Cell death & disease; 10(10): 1-12.
43. Zhang H, Zhang J, Han Q, et al. (2020): Progranulin as a novel biomarker for treatment response and prognosis in patients with MM. Cancer Manag Res;12:667-675.