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Progranulin/ Tumor Necrosis Factor-α Ratio in Rheumatoid Arthritis

Rabab Hamdy Mohamed [a*], Enayat Ali Badr [a], Samy Abd-el-Kader Khoder [b] and Mohamed Kamal Zahra [a]

[a] Clinical Pathology, Faculty of Medicine, Tanta University, Tanta, Egypt.
[b] Internal Medicine, Faculty of Medicine, Tanta University, Tanta, Egypt.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Progranulin (PGRN) has significant functions in several processes, including immune response and the development of cancer. This study aims to research the relationship between the ratio of serum progranulin/tumor necrosis factor (TNF) alpha and the rheumatoid arthritis (RA) activity.
Methods: This study included 35 patients with rheumatoid arthritis whom we collected their complete history with personal and family history included in addition to 30 seemingly healthy individuals as a normal control group. In addition to clinical examinations, laboratory tests, and serum progranulin level and TNF-alpha by the Enzyme-linked Immunosorbent Assay (ELISA).
Results: There were significant increases in Erythrocyte Sedimentation Rate (ESR), C-reactive protein (CRP), Rheumatoid Factor (RF), PGRN and TNF alpha in the diseased group compared to the control group. The serum levels of PGRN and TNF alpha in the rheumatoid arthritis community were significantly higher than in the control group. In the RA community, there was a positive association between the PGRN/TNF alpha ratio and Disease Activity Score in 28 joints (DAS28) score and RF. The relationship between PGRN and the severity ratio was significant. The relationship between TNF alpha and the severity ratio of the disease were highly significant. Also, a significant relationship between the PGRN/TNF alpha ratio and DAS28 score was significant.
Conclusion: An important correlation between circulating levels of PGRN and TNF-alpha, and activity of the disease was in RA patients.

**Corresponding author: E-mail: thesismse@gmail.com;*

Keywords: Rheumatoid arthritis; progranulin; Tumor Necrosis Factor-Alfa.

1. INTRODUCTION

In rheumatoid arthritis (RA), TNF alpha is attributable to inflammation and destruction of joints. Nineteen ligands and 35 receptors have TNF cytokines. Endothelial and immune cells express TNF alpha. Metalloproteinase cleaves TNF alpha and works with 2 receptors (TNFR1&TNFR2) [1].

Progranulin is a growth factor that is involved in wound cure, inflammation, infection, and tumorigenesis. It competes with tumor necrosis factor (TNF) alpha receptors, thus inducing anti-inflammatory function [2].

There is an exceptionally wide range of biological activities of tumor necrosis factor (TNF). Cytotoxicity to tumor cell lines was one of the first functions to be identified that resulted in its name tumor necrosis factor. TNF-alpha is produced predominantly by monocytes and macrophages, but also by fibroblasts, T-cells and B-cells. It is one of the main molecules of cytokines that induces inflammation in RA.

This study aims to research the relationship between the ratio of serum progranulin/tumor necrosis factor alpha and the RA activity.

2. PATIENTS AND METHODS

The present study was carried out on 35 patients with RA diagnosed on the basis of their clinical manifestations and laboratory diagnosis. In addition, the study includes 30 healthy individuals with matched sex and age served as control group. They were recruited from the outpatient clinic of rheumatology department police hospital Alexandria during the period from October 2017 to March 2018.

The patients of RA included 16 females and 19 males. Their age ranged from 50 to 70 years. RA lesions were examined and the following was recorded: DAS28 score was calculated for each patient as an overall determinant of severity. The control group included 30 normal health persons their aged ranged from 40 to 60 years.

The patients of this study were classified according to disease severity: DAS28 score divide the patients into: Group 1: mild severity group includes twelve patients represent of the studied patients they were (34.3%) 6 females and 6 males with DAS28 score_<3.2 and >2.6. And Group 2: moderate severity group includes eleven patients represent of the studied patients they were (31.4%) 4 females and 7 males with DAS28 >3.2 and_<5.1. And Group 3: severe group includes 12 patients represent of the studied patients they were (34.3%) 6 females and 6 males with DAS28 >5.1.

All patients of other autoimmune diseases were excluded from the study. And all patients had to present personal history with name, age, occupation, residency and special habits. As well as full clinical examination, general clinical examination, laboratory investigations such as complete blood picture, serological tests for RA, hepatic and renal functions tests, in addition to the determination of progranulin and TNFα.

Seven millimeters of venous blood were collected from each subject by use of disposable sterile syringes under complete aseptic conditions. A sum of 2 ml blood was collected on ethylene di amine tetra acetic acid (EDETA) tube and mixed thoroughly to perform complete blood count. The remaining blood was collected on a plain tube allowed to pass gently along the wall of a clean dry plain test tube and allowed to clot for 10-20 minutes at room temperature before centrifugation. Then serum was separated and divided into three aliquots, two was stored at -20ºC to be used for estimation of serum progranulin and TNFα and the third aliquot was used for other routine investigations. The ELISA assay employs the quantitative sandwich enzyme immunoassay technique. Monoclonal antibodies specific for PGRN or TNF-α have been pre-coated onto a microplate. Standards and samples were added into the wells and any PGRN or TNF-α were bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for PGRN or TNF-α was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of PGRN or TNF-α bound in the initial step. The color development was stopped and the intensity of the color was measured by spectrophotometric reader.

2.1 Statistical Analysis

Statistical analysis was done by SPSS v25 (IBM©, Chicago, IL, USA). Quantitative data

were presented as mean, standard deviation (SD) median and interquartile range (IQR). Quantitative data were analysed by unpaired student t-test, Mann Whitney-test or ANOVA test. Qualitative data were presented as number and percent and were compared by chi-square or Fisher's Exact test when appropriate. ROC-curve was used to show the diagnostic accuracy of markers. A two tailed P value <0.05 was considered statistically significant.

3. RESULTS

Table 1 shows that the difference between the mean serum level of Erythrocyte Sedimentation Rate (ESR), C-reactive protein (CRP) in both control and patients group was statistically highly significant (p value<0.001). Moreonver, regarding the Neutrophil/Lymphocytes Ratio (N/L Ratio), the difference between both control and patients group was statistically highly significant (p value 0.001).

Table 2 shows that the difference between serum levels of PGRN and TNF-α in both control and patients' groups was statistically highly significant (p value <0.001). the serum level of PGRN/TNF-α ratio which appears to be statistically highly significant (p value<0.001).

Table 1. Comparison between the two studied groups according to routine laboratory tests

Test		Control (n = 30)	Patients (n = 35)	Test of sig.	P
ESR (mms/hr)	Min. – Max.	15.0 - 40.0	18.0 - 110.0	U= 118.0*	<0.001*
	Mean ± SD.	25.37 ± 7.43	65.26 ± 32.46		
	Median (IQR)	25.0 (20.0 - 30.0)	70.0 (30.0 - 105.0)		
CRP (mg/l)	Min. – Max.	1.50 - 3.80	24.0 - 30.0	t= 73.291*	<0.001*
	Mean ± SD.	2.66 ± 0.61	25.63 ± 1.73		
	Median (IQR)	2.50 (2.20 - 3.0)	25.0 (24.50 - 26.0)		
Neutrophils %	Min. – Max.	56.50 - 70.0	45.0 - 75.0	t= 1.512	0.136
	Mean ± SD.	64.56 ± 4.82	62.07 ± 8.26		
	Median (IQR)	65.75 (59.60 - 69.30)	63.30 (54.65 - 69.50)		
Lymphocytes%	Min. – Max.	26.20 - 38.0	16.80 - 41.30	t= 1.498	0.141
	Mean ± SD.	30.36 ± 3.27	28.29 ± 7.35		
	Median (IQR)	29.65 (28.0 - 32.20)	25.50 (23.05 - 35.10)		
N/L ratio	Min. – Max.	1.50 - 2.50	0.70 - 6.07	U= 197.00*	<0.001*
	Mean ± SD.	1.89 ± 0.27	3.22 ± 1.45		
	Median (IQR)	1.85 (1.70 - 2.10)	3.0 (2.10 - 4.15)		

**significant as P value <0.05. ESR: Erythrocyte Sedimentation Rate, CRP: C-reactive protein, N/L Neutrophil/Lymphocyte Ratio, t: Student T test, U: Mann-Whitney test*

Table 2. Comparison between the two studied groups according to PGRN, TNF-α levels and PGRN/TNF-α ratio

Parameter		Control (n = 30)	Patients (n = 35)	T	P
PGRN level (ng/ml)	Min. – Max.	20.0 – 47.0	48.0 – 64.0	15.275*	<0.001*
	Mean ± SD.	33.73 ± 6.07	54.79 ± 5.04		
	Median (IQR)	35.0 (33.0 – 36.0)	55.0 (49.75 – 58.50)		
TNF-α level (pg/ml)	Min. – Max.	2.40 – 4.20	59.0 – 110.0	27.436*	<0.001*
	Mean ± SD.	3.44 ± 0.58	81.26 ± 16.77		
	Median (IQR)	3.55 (2.90 – 4.0)	79.0 (68.50 – 97.0)		
PGRN/TNF-α ratio	Min. – Max.	4.76 – 16.79	0.49 – 0.88	18.895*	<0.001*
	Mean ± SD.	10.17 ± 2.75	0.69 ± 0.10		
	Median (IQR)	10.57 (8.95 – 12.0)	0.70 (0.61 – 0.77)		

**Significant as P value <0.05. Progranulin (PGRN), Tumor Necrosis Factor - Alpha (TNFα)*

Table 3 shows the relation between PGRN and type severity ratio in patients group as in the mild group serum PGRN level with a mean of 50.38 ± 1.76ng/ml which is highly significant (p<0.001), moderate severity group with a mean standard of 54.36 ± 3.75ng/ml which is highly significant (p<0.001) while in severe group the mean standard was 59.58 ± 4.01ng/ml with highly significant (p<0.001) which showing that serum PGRN increase with the severity of the disease. the relation between TNF-α and type severity ratio in patients group as in mild group the mean standard 64.08 ± 4.50pg/ml with a high significant (p<0.001), moderate group with a mean standard of 77.82 ± 5.08pg/ml while in the severe group there is increasing mean standard of 101.6 ± 6.40pg/ml which show a high significant increase of serum TNFα with the severity of the disease (p<0.001).the relation between PGRN/TNF-α ratio and type severity ratio in patients group as it was the highest in the mild group with a mean standard of 0.79±0.06 and decrease at moderate group with mean standard of 0.70±0.07 showing the lowest mean standard at the severe group 0.59±0.05 with a highly significant decreasing with the severity (p<0.001).

Table 4 shows no significant between control and patients' groups. Also, the difference of significance between the patient and control group was insignificant (p value 0.370). Yet, the relation between PGRN and type severity ratio in patients group as in the mild group serum PGRN level with a mean of 50.38 ± 1.76ng/ml which is highly significant (p<0.001), moderate severity group with a mean standard of 54.36 ± 3.75ng/ml which is highly significant (p<0.001) while in severe group the mean standard was 59.58 ± 4.01ng/ml with highly significant (p<0.001) which showing that serum PGRN increase with the severity of the disease. The relation between TNF-α and type severity ratio in patients group as in mild group the mean standard 64.08 ± 4.50pg/ml with a high significant (p<0.001), moderate group with a mean standard of 77.82 ± 5.08pg/ml while in the severe group there is increasing mean standard of 101.6 ± 6.40pg/ml which show a high significant increase of serum TNFα with the severity of the disease (p<0.001). The relation between PGRN/TNF-α ratio and type severity ratio in patients' group as it was the highest in the mild group with a mean standard of 0.79±0.06 and decrease at moderate group with mean standard of 0.70±0.07 showing the lowest mean standard at the severe group 0.59±0.05 with a highly significant decreasing with the severity (p<0.001).

There were significant strong correlation between PGRN/TNF-α ratio and both DSA28 and RF score in patients' group (r = -0.746 and -0.694 respectively, p<0.001).

Table 5 and Figs. 1 and 2 expressed a sum of different parameters on the levels of PGRN and TNG-Alpha with sensitivity, specificity as well as PPV8 and NPPV in regard to both the mild and seevre groups of subjects.

Table 3. Relation between type severity ratio and different parameters according to DAS28score

Parameter		Type severity ratio			F	P
		Mild (2.6 : 3.1)	Moderate (3.5 : 4.9)	Severe (5.4 : 8.0)		
PGRN level (ng/ml)	Min. – Max.	48.0 – 54.0	49.0 – 60.0	49.0 – 64.0	23.287*	<0.001*
	Mean ± SD.	50.38 ± 1.76	54.36 ± 3.75	59.58 ± 4.01		
	Median	50.0	55.0	59.50		
TNF-α level (pg/ml)	Min. – Max.	59.0 – 70.0	70.0 – 85.0	90.0 – 110.0	148.19*	<0.001*
	Mean ± SD.	64.08 ± 4.50	77.82 ± 5.08	101.6 ± 6.40		
	Median	65.0	79.0	101.0		
PGRN/TNF-α Ratio	Min. – Max.	0.70 – 0.88	0.62 – 0.83	0.49 – 0.65	37.469*	<0.001*
	Mean ± SD.	0.79 ± 0.06	0.70 ± 0.07	0.59 ± 0.05		
	Median	0.78	0.70	0.61		

**Significant as P value <0.05. Progranulin (PGRN), Tumor Necrosis Factor - Alpha (TNFα)*

Table 4. Relation between type severity ratio and different parameters

Parameter		Type severity ratio			#Control (n = 30)	Test of Sig.	p
		Mild (n = 12)	Moderate (n = 11)	Severe (n = 12)			
Total leukocyte count10^^{3}/mm^3	Min. – Max.	4.0 – 8.0	3.0 – 10.0	5.20 – 12.0	4.70 – 10.30	F=	0.051
	Mean ± SD.	5.92 ± 1.62	6.85 ± 2.07	8.01 ± 2.30	7.52 ± 1.68	3.260	
	Median	5.50	7.0	7.0	7.75		
Neutrophils%	Min. – Max.	48.30 – 70.0	45.0 – 70.0	59.30 – 75.0	56.50 – 70.0	F=	0.002*
	Mean ± SD.	57.73 ± 7.55	60.05 ± 8.22	68.26 ± 5.12	64.56 ± 4.82	7.347*	
	Median	57.30	60.0	69.75	65.75		
Lymphocytes%	Min. – Max.	20.90 – 41.30	16.80 – 38.20	18.20 – 38.20	26.20 – 38.0	F= 1.380	0.266
	Mean ± SD.	30.94 ± 7.61	25.99 ± 7.55	27.75 ± 6.64	30.36 ± 3.27		
	Median	30.55	25.0	25.85	29.65		
N/L ratio	Min. – Max.	1.50 – 3.10	0.70 – 4.20	2.50 – 6.07	1.50 – 2.50	H=	0.005*
	Mean ± SD.	2.43 ± 0.61	2.72 ± 1.27	4.47 ± 1.42	1.89 ± 0.27	10.773*	
	Median	2.45	2.80	5.29	1.85		
ESR (mms/hr)	Min. – Max.	25.0 – 110.0	30.0 – 110.0	18.0 – 110.0	15.0 – 40.0	H=	0.168
	Mean ± SD.	52.92 ± 31.22	79.09 ± 30.15	64.92 ± 33.13	25.37 ± 7.43	3.572	
	Median	35.0	70.0	70.0	25.0		
CRP mg/l	Min. – Max.	24.0 – 30.0	24.0 – 30.0	24.0 – 27.0	1.50 – 3.80	F=	0.055
	Mean ± SD.	25.33 ± 1.72	26.64 ± 2.11	25.0 ± 0.85	2.66 ± 0.61	3.184	
	Median	25.0	26.0	25.0	2.50		
RF iu/ml	Min. – Max.	8.0 – 32.0	16.0 – 32.0	64.0 – 128.0	–	H=	<0.001*
	Mean ± SD.	16.0 ± 8.36	26.18 ± 8.07	96.0 ± 33.42	–	26.899*	
	Median	16.0	32.0	96.0	–		
Anti CCP (mg/dl)	Min. – Max.	18.0 – 22.0	17.0 – 22.0	17.50 – 22.0	–	F=	0.370
	Mean ± SD.	20.58 ± 1.62	19.55 ± 2.07	20.21 ± 1.56	–	1.024	
	Median	21.0	18.0	20.0	–		
ANA	Min. – Max.	10.0 – 20.0	10.0 – 20.0	10.0 – 20.0	10.0 – 20.0	H=	0.979
	Mean ± SD.	14.17 ± 5.15	14.55 ± 5.22	14.17 ± 5.15	14.67 ± 5.07	0.043	
	Median	10.0	10.0	10.0	10.0		
PGRN level(ng/ml)	Min. – Max.	48.0 – 54.0	49.0 – 60.0	49.0 – 64.0	20.0 – 47.0	F=	<0.001*

	Mean ± SD.	50.38 ± 1.76	54.36 ± 3.75	59.58 ± 4.01	33.73 ± 6.07	23.287*	
	Median	50.0	55.0	59.50	35.0		
TNF-α level(pg/ml)	Min. – Max.	59.0 – 70.0	70.0 – 85.0	90.0 – 110.0	2.40 – 4.20	F=	<0.001*
	Mean ± SD.	64.08 ± 4.50	77.82 ± 5.08	101.6 ± 6.40	3.44 ± 0.58	148.19*	
	Median	65.0	79.0	101.0	3.55		
PGRN/TNF-αratio	Min. – Max.	0.70 – 0.88	0.62 – 0.83	0.49 – 0.65	4.76 – 16.79	F=	<0.001*
	Mean ± SD.	0.79 ± 0.06	0.70 ± 0.07	0.59 ± 0.05	10.17 ± 2.75	37.469*	
	Median	0.78	0.70	0.61	10.57		

** significant as P value <0.05. Neutrophil/Lymphocyte Ratio (N/L), Erythrocyte Sedimentation Rate (ESR), C-reactive protein (CRP), Rheumatoid Factor (RF), Anti Cyclic Citrullinated Peptide antibodies (CCP), Antinuclear Antibody (ANA), Progranulin (PGRN), Tumor Necrosis Factor - Alpha (TNFα)*

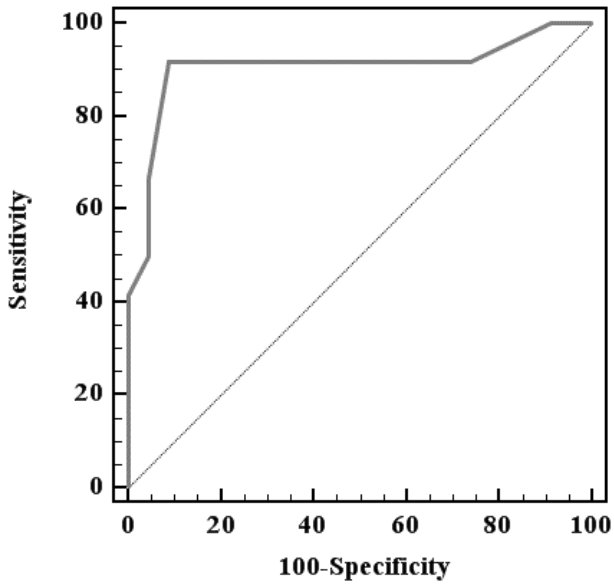

Fig. 1. ROC curve for PGRN level to diagnose severe patients (n= 12/35)

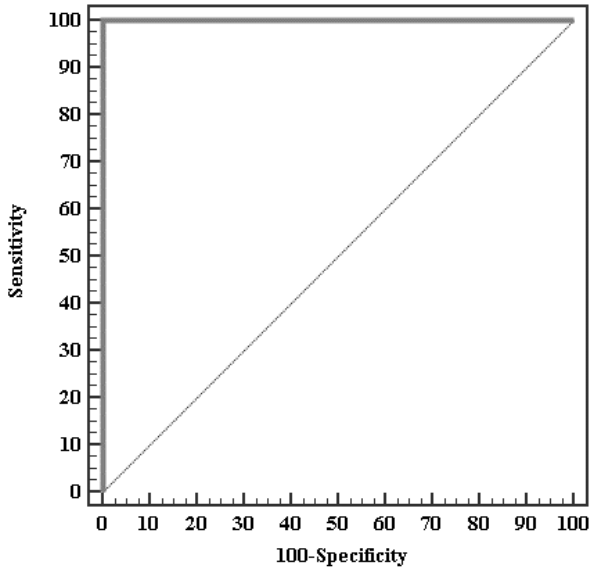

Fig. 2. ROC curve for TNF-α level to diagnose sever patients (n= 12/35)

Table 5. Agreement (sensitivity, specificity) for PGRN and TNF-α levels to diagnose patients with mild and severe disease degrees (n= 12/35)

Parameter		AUC	P	95% C.I	Cut off	Sensitivity	Specificity	PPV	NPV
PGRN level	Mild	0.862	0.001*	0.733 – 0.992	≤52	91.67	82.61	73.3	95.0
	Severe	0.906	<0.001*	0.771 – 1.040	>56#	91.67	91.30	84.6	95.5
TNF-α level	Mild	0.993	<0.001*	0.975 – 1.011	≤70#	100.0	91.30	85.7	100.0
	Severe	1.000	<0.001*	1.000 – 1.000	>85#	100.0	100.0	100.0	100.0

4. DISCUSSION

PGRN binds to receptors for tumor necrosis factor (TNFR) and thus restricts the function of TNF in inflammatory arthritis. By inhibiting ADAMTS-7/ADAMTS-12-mediated COMP degradation, PGRN also strongly suppresses cartilage destruction and thus plays a significant role in preventing joint destruction in arthritis [3].

Our findings were comparable to the findings of Peng et al. [4] who found that RF and CRP and ESR are improved in patients with RA than the apparently stable control group. Though neutrophils, lymphocytes, ANA and total leukocyte count were not different between the two control groups and the patient group.

We discovered that the serum PGRN level is significantly higher in RA patients than in the stable control group, coinciding with the findings recorded by Yamamoto et al. [5] who found serum PGRN to be much higher than the moderate and mild severity of the disease in RA patients with DAS28 >5.1.

Vasanthi et al. [6] reported that there was a rise in serum TNF alpha in RA disease, which was also shown in the current study that Vasanthi clarified that people with RA have high TNF alpha levels in the synovial fluid and that significantly contribute in inflammation and joint destruction, which are characteristics of RA, and studies have shown dramatic response.

We found a clear significant association between PGRN and the severity ratio that correlated with Cerezo et al. [7], confirming that PGRN in RA disease was associated with disease activity. There was also a clear significant correlation between TNF alpha and disease severity ratio that associated with Walters HM et al. [8]. Our PGRN/TNF-alpha ratio was also correlated with the stage of the disease in RA patients.

5. CONCLUSION

This study indicates an important correlation between circulating levels of PGRN and TNF-alpha, an activity of the disease in RA patients. These results need further investigations with respect to PGRN and TNF-α in RA.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

The study was done after approval from the Ethical Committee of Faculty of Medicine, Tanta University and obtaining written informed consent.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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