ORIGINAL ARTICLE

Diagnostic Utility of Peptidoglycan Recognition Protein 2 (PGLYRP2) in Systemic Lupus Erythematosus Patients Attending Benha University Hospitals

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ABSTRACT

Background: *PGLYRP2 is essential for innate immune responses and contributes to the progression of chronic inflammation. Elevated PGLYRP2 levels can result in prolonged inflammation. So in individuals with SLE, the concentration of PGLYRP2 in the blood rises significantly and is linked to a higher SLEDAI score.* ***Objectives****: This work was conducted to assess the PGLYRP2 level in the SLE patient's serum and study its association with clinical manifestations, laboratory parameters, disease activity and organ damage.* ***Methodology****: twenty-five SLE patients' blood samples were obtained, leucocytes were isolated and PGLYRP2 expression was evaluated by RT-PCR then serum PGLYRP2 levels were detected by ELISA. Twenty-five healthy participants, selected to match in age and gender, were designated as the control group.* ***Results****: PGLYRP2 expression level in the leucocytes was higher in the SLE group than in the healthy control group ($p< 0.05$). The gene expression in the cases group was relatively higher. Serum PGLYRP2 levels were significantly higher in cases with SLE with range between (1.34-5.73) ng /mL ($p< 0.05$).* ***Conclusion****: Our results support the hypothesis that PGLYRP2 contributes in the development of the pathogenesis of SLE, activity of disease. PGLYRP2 appears to serve as a useful marker with good sensitivity.*

INTRODUCTION

Systemic lupus erythematosus (SLE) is a long-term autoimmune disorder with a complicated and incompletely understood pathogenesis [1].

SLE is recognized as one of the most difficult and defy disorders in medicine, distinguished by immune system dysfunction [2]. The cause of SLE involves multiple factors, including both environmental influences and genetic predispositions. SLE impacts multiple organs, with the kidneys, joints, skin, lungs, blood components, and central nervous system being most commonly affected [3].

Peptidoglycan recognition proteins (PGRPs) were first identified in the blood of the Bombyx mori and then found in mammals, including humans and mice. More than 100 different members of the PGLYRP family have been recognized. These proteins are highly conserved and typically contain one or more PGLYRP domain, which resembles the N-acetylmuramic acid-L-alanine amidase enzyme[4].

Peptidoglycan recognition protein 2 (PGLYRP2) works as a pattern recognition receptor that produces a protein capable of identifying peptidoglycan, an essential element of bacterial cell walls. It is predominantly found in the liver and is essential for modulating the body's innate immune responses [5].

PGLYRP2 in humans is an enzyme that relies on zinc and acts as an N-acetylmuramoyl-L-alanine amidase. It cleaves the bond between N-acetylmuramic acid and L-alanine in bacterial peptidoglycan. The smallest peptidoglycan fragment that PGLYRP2 can hydrolyze is the N-acetylmuramic acid-tripeptide. However, PGLYRP2 does not have direct bacteriolytic activity [6].

PGLYRP2 plays a various kinds of roles, including infectious diseases, immune responses, tumor progression, inflammation, and development of brain [7,8].

PGLYRP2 is crucial for the activation of cytokines, chemokines, and receptors in an arthritis model [9]. It has been described that PGLYRP2 links the innate immune response to the adaptive immune response, functioning as a scavenger that breaks down bioactive peptidoglycan (PGN) into biologically inactive fragments and modulates NOD1/2-mediated inflammation which are intracellular pattern recognition receptors and act as sensor of bacterial peptidoglycans[10].

The aim of this work is to measure PGLYRP2 level in the SLE patient's serum and to study its association with clinical manifestations, laboratory parameters, disease activity and organ damage.

METHODOLOGY

Our study is a case-control study involved 25 SLE patients who met the Systemic Lupus International Collaborating Clinics (SLICC) classification criteria for the disease [11]. The lupus ages ranged between 19-49 years with mean of 28.24±8.38 years, they were 19 females (76%) & 6 males (24%). The patients were chosen from the inpatient and outpatient clinics of the Rheumatology, Rehabilitation, and Physical Medicine Department at Benha University Hospitals, between October 2023 and June 2024.

A total of 25 apparently healthy volunteers, matched for age and sex, participated as a control group. The practical aspects of this study were conducted at the Department of Medical Microbiology and Immunology, Faculty of Medicine, Benha University.

All patients underwent a full history taking, well clinical examination and laboratory tests, including hemoglobin (Hb), white blood count (WBC), platelet count, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), antinuclear antibodies (ANA), anti-dsDNA antibody titers, C3 and C4 levels, serum creatinine, blood urea, and protein analysis from 24-hour urine collections.

Assessment of the activity of disease was done by Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score as following: no activity (SLEDAI = 0), mild activity (SLEDAI = 1 to 5), moderate activity (SLEDAI = 6 to 10), high activity (SLEDAI = 11 to 19) and Very high activity (SLEDAI ≥20) [12].

Assessment of organ damage by the Systemic Lupus International Collaborating Clinics (SLICC) damage index [13].

All participants signed a written consent form after being fully informed about the study before their involvement.

Exclusion criteria

Patients with Age <18 years , pregnant women, smokers, who have other rheumatic autoimmune diseases, cancers, or infectious diseases and who were out of a normal Body mass index (BMI) range.

Approval for the study design was acquired from the Ethics Committee, Faculty of Medicine, Benha University (Number: MD 14-12-2021)

PGLYRP2 analysis

PGLYRP2 expression by Real-time PCR

We collected 3 ml of venous blood from each participant for the purposes of RNA extraction and serum analysis.

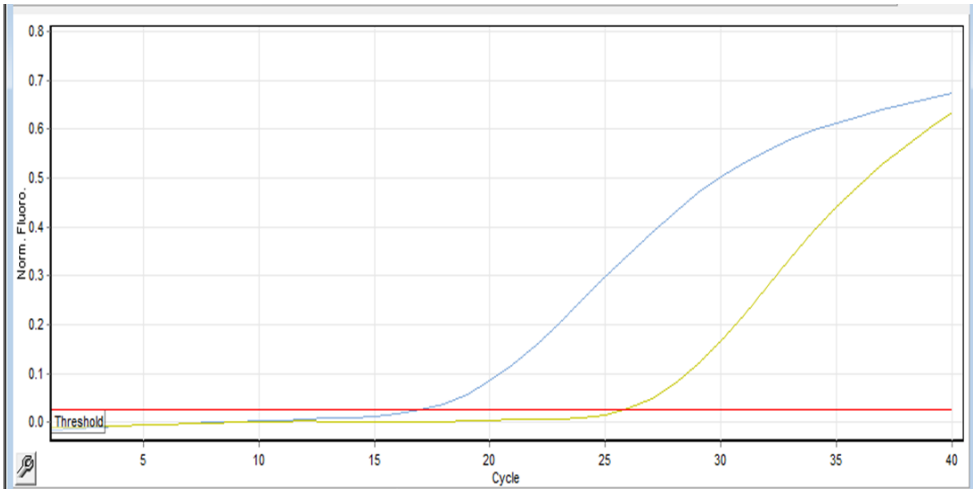
Peripheral blood mononuclear cells (PBMCs) were isolated then RNA was extracted by using QIAamp RNA Blood Mini Kit (**QIAGEN ®; Germany**) according to manufacturer's instructions[14]. Complementary DNA (cDNA) was prepared by using Ipsogen Reverse Transcription Kit (**QIAGEN ®; Germany**) according to manufacturer's instructions. The reverse transcription program was run by using a thermo cycler as following Settings (reverse transcription at 25°C for 10 minutes then at 50°C for 60 minutes, inactivation 85°C for 5 minutes and cooling at 4°C for 5 minutes)[15]

Real-time PCR was done to measure the relative expression level of PGLYRP2 by QuantiNova SYBR Green PCR Kit (**QIAGEN ®; Germany**) according to manufacturer's instructions. Primers for PGLYRP2 were used from (**MACROGEN®; South Korea**) [16] (table1).

Table 1: Primers Sequences used in the study [8]

Name	Sequences 5 → 3
PGLYRP2 forward primer	5′-GCA CTT CAC CGC GAC TGT TA-3′
PGLYRP2 reverse primer	5′-TTA TTG GAG GTC TGT GGC TGG-3′
GAPDH forward primer	5′-TCA GTG GTG GAC CTG ACC TG-3′
GAPDH reverse primer	5′-TGC TGT AGC CAA ATT CGT TG-3′

Amplification was done on a Rotor-Gene Q realtime PCR machine **(Qiagen; Germany**) using the following PCR thermal cycle conditions: initial hold at 95°C for 2 minutes, followed by 40 cycles, including denaturation for 5 seconds at 95°C and annealing at 62°C for 10seconds. Relative expression level of PGLYRP2was evaluated by the 2ˆ–delta Cycle threshold ($2^{-\Delta\Delta CT}$) method, with GAPDH as the internal reference. Then melting curve analysis of the PCR was performed as shown in (figure1).



Ct GAPDH=17.07 **Ct PGLYRP2 =25.82**

Fig. 1: The threshold line and Cycle threshold (Ct) of a sample of lupus patient.

PGLYRP2 serum detection by enzyme-linked immunosorbent assay test (ELISA)

Serum level of PGLYRP2 was measured using with ELISA kits (**ELK Biotechnology ®; China)** according to manufacturer's instructions. 7 wells for standards and 1 for Blank were prepared.100 µL of each standard working solution and samples were added to the appropriate wells. 100 µL of diluted Biotinylated Antibody was added to each well and incubated for 50 minutes at 37°C. 100 µL of Streptavidin-HRP Working Solution was applied to each well, a 90 µL of TMB Substrate Solution was applied to each well and incubated for 20 minutes at 37°C in the dark. The microplate reader was preheated for 15 minutes before measuring optical density (OD). 50 µL of stop reagent was added, turning the solution yellow. OD was measured at 450 nm, using the blank well as the zero reference[17].

RESULTS

The lupus ages ranged between 19-49 years with mean of 28.24±8.38 years, they were 19 females (76%) & 6 males (24%).

The SLEDAI score ranged between **2** and **24**. As regard the activity of the disease among studied lupus cases; there were **5** cases (**20**%) had mild activity (**SLEDAI between 1 to 5**) , **10** cases (**40**%) had moderate activity (**SLEDAI between 6 to 10**) , **7** cases (**28**%) had high activity (**SLEDAI between 11 to 19**) and **3** cases (**12**%) had Very high activity (**SLEDAI ≥20**)

Table 2: Comparison of fold change and ELISA between cases and control groups

	Cases group N=25	**Control group N=25**	**Significance**
Fold change = $2^{-\Delta\Delta Ct}$	1.01(0.03-32.9)	0.401(0.0-12.3)	z=2.42 p=0.016*
ELISA ng/m	3.84(1.34-5.73)	1.5(0.03-5.09)	Z=3.71 P=0.001*

Z= Mann Whitney U test , *****statistically significant

There was up regulation of PGLYRP2 expression level in the leucocytes. The level was higher in the SLE group than in the healthy control group with significant statistical value (p< 0.05). In our study there was statistically significant difference between SLE cases and the control group regarding serum PGLYRP2 as it was significantly higher in cases with SLE with range between (1.34-5.73) ng /mL (p< 0.05) (Table 2) .

As regard relation between disease activity and demographic characters of studied lupus cases; there were **2** males (**40**%) and **3** females (**60**%) had mild activity, **3** males **(30%)** and **7** females **(70%)** had moderate activity and **1** male **(10%), 9** females **(90%)** had high and very high activity.

Table 3: Relation between disease activity and demographic characters of studied cases

	Disease activity			Significance
	Mild	**Moderate**	**High / very high**	
Age / years	25.20±7.08	26.0±5.25	32.0±10.53	F=1.80 P=0.188
Sex Males Females	 2(40) 3(60)	 3(30) 7(70)	 1(10) 9(90)	 MC=1.97 P=0.373

F:One Way ANOVA test , **MC**: Monte Carlo test

There was No statistically significant difference in age and gender across different disease activity scores (p>0.05) (Table 3).

Table 4: Relation between disease activity, medical history and clinical presentation among studied cases

	Disease activity			Significance
	Mild	**Moderate**	**High / very high**	
DM	0	0	1(10)	MC=1.56 P=0.458
Hypertension	3(60)	0	2(20)	MC=7.5 P=0.024*
Skin rash	2(40)	8(80)	6(60)	MC=2.43 P=0.297
Arthralgia	0	8(80)	7(70)	MC=9.58 P=0.008*
Alopecia	0	5(50)	6(60)	MC=5.11 P=0.078
Oral ulcers	4(80)	8(80)	6(60)	MC=1.19 P=0.551
Eye	1(20)	5(50)	2(20)	MC=2.48 P=0.289
CNS	0	0	3(30)	MC=5.11 P=0.078
Vasculitis	3(60)	3(30)	4(40)	MC=1.25 P=0.535
Chest	2(40)	2(20)	5(50)	MC=1.99 P=0.369
CVS	2(40)	3(30)	6(60)	MC=1.87 P=0.393
FMS	1(20)	3(30)	2(20)	MC=0.329 P=0.848
Renal	5(100)	1(10.0)	3(30)	MC=11.98 P=0.003*
Organ damage	0	1(10)	5(50)	MC=6.36 P=0.042*

Significant differences (p<0.05) were found between mild, moderate high and very high cases regarding hypertension **(P=0.024)** which was more prevalent in the mild disease activity group, arthralgia (**P=0.008**) which was more prevalent in the moderate disease activity group, renal symptoms (P**=0.003**) which was more prevalent in the mild disease activity group and organ damage which was more prevalent in the high/very high activity group (Table 4).

Table 5: Correlation between PGLYRP2 fold increase and demographic, clinical, PGLYRP2 serum level and laboratory findings of studied cases.

	Fold increase	
	r	**p**
Age / years	0.014	0.949
Disease duration	-0.109	0.602
Number of affected joints	-0.423	0.117
HB	0.025	0.905
TLC	-0.220	0.291
Platelet	0.289	0.161
ESR	-0.308	0.135
ALT	-0.140	0.505
AST	-0.076	0.717
Urea	-0.136	0.526
Creatinine	-0.321	0.117
Uric acid	-0.119	0.581
Urine protein (mg/dl)	-0.01	0.961
Na	0.226	0.278
K	0.316	0.124
Serum Ca	0.049	0.816
Albumin	0.116	0.582
SLEDAI	0.173	0.409
Organ damage	-0.111	0.599
Serum PGLYRP2	0.159	0.529

r: Spearman correlation coefficient

There were statistically non-significant correlations between fold increase and age, disease duration, number of affected joints, hemoglobin, TLC, Platelet Count, ESR, ALT, AST, urea, creatinine, uric acid, urine protein, Na, K, serum Ca, albumin , SLEDAI score, Organ damage and Serum PGLYRP2 ($p>0.05$)(Table 5).

Table 6: Correlation between serum PGLYR

	Serum PGLYRP2 levels by ELISA ng/ml	
	r	P
Age / years	-0.464	0.052
Disease duration	-0.138	0.586
Number of affected joints	-0.180	0.596
Renal damage	0.169	0.504
HB	0.439	0.07
TLC	0.097	0.702
Platelet	0.061	0.810
ESR	-0.126	0.620
ALT	-0.155	0.540
AST	0.299	0.228
Urea	-0.127	0.627
Creatinine	-0.153	0.545
Uric acid	-0.07	0.788
C3	-0.485	0.04*
C4	-0.482	0.043*
Urine protein (mg/dl)	-0.133	0.611
Na	-0.283	0.255
K	-0.042	0.869
Serum Ca	-0.009	0.971
Albumin	0.298	0.229
SLEDAI	0.491	0.038*
Organ damage	-0.361	0.142

P2 levels by ELISA and demographic , clinical and laboratory findings of studied cases.

r: Spearman correlation coefficient, ***statistically significant

There were statistically non-significant correlations between age, disease duration, number of affected joints, renal damage , hemoglobin, TLC, Platelet Count, ESR, ALT, AST, urea, creatinine, uric acid, urine protein, Na, K, serum Ca, albumin and organ damage and serum PGLYRP2 levels by ELISA (p>0.05) (Table 6).

There were statistically significant correlation between C3, C4 and SLEDAI score and serum PGLYRP2 levels by ELISA (p<0.05) (Table 6).

Table 7: Validity of fold change & serum PGLYRP2 by ELISA in differentiating cases from control groups

	AUC (95% CI)	P value	Cut off point	Sensitivity %	Specificity %
Serum PGLYRP2 by ELISA	0.841 (0.719-0.962)	0.001*	≥2.91	82.6	72.2
Fold change of PGLYRP2	0.699 (0.553-0.845)	0.016*	≤0.973	84.0	52.0

AUC: Area under curve , **CI**: Confidence interval *****statistically significant

Area under the Curve (AUC) for fold increase of PGLYRP2 was 0.699, While AUC for Serum PGLYRP2 by ELISA was 0.841 indicating a moderate ability to discriminate between groups

Fold increase of PGLYRP2 have a significant p-value (0.016), meaning the discriminative ability is statistically significant (p<0.05). Serum PGLYRP2 also is statistically significant (p<0.05).

Fold increase of PGLYRP2 has a sensitivity of 84.0% and specificity of 52.0%. (Table 7) (figure 2).

Serum PGLYRP2 has a sensitivity of 82.6% and specificity of 72.2%. (Table 7) (figure 3).

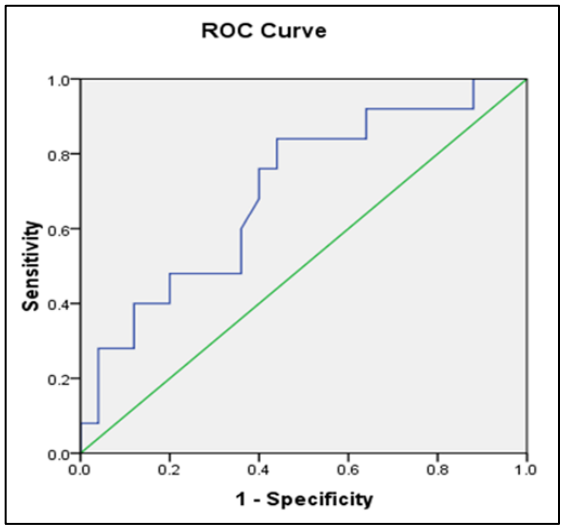


Fig. 2: ROC curve of fold increase of PGLYRP2in differentiating cases from control group

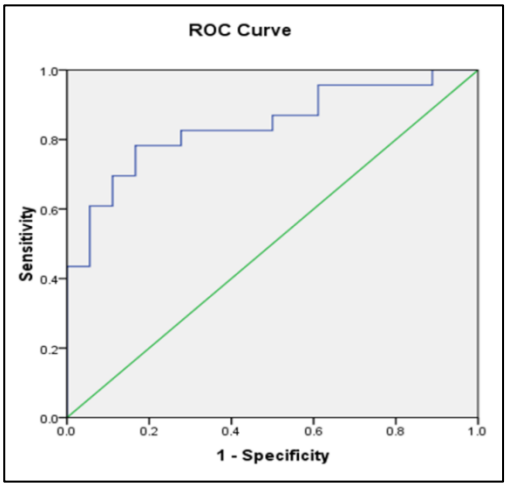


Fig. 3: ROC curve of serum PGLYRP2 by ELISA in differentiating cases from control group

DISCUSSION

The expression level of PGLYRP2 in the leucocytes was higher in the SLE group than in the healthy control group (p< 0.05). There was also a statistically significant difference between SLE patients and the control group regarding delta Ct values (p< 0.05).This suggests that the gene expression in the cases group is relatively higher. This could be attributed to that PGLYRP-2 directly enhances cell responsiveness to cytokines [9] and it is known that the modulation of these cytokines may impact SLE progression[18]. In agreement to our study Li et al [8] evaluated the level of PGLYRP2 gene expression among patients with SLE. Their study demonstrated that the expression level of PGLYRP2 was elevated in SLE patients, moreover, was greater in active LN patients compared to healthy volunteers.

In contrast to our findings, Swaminathan [19] noted that that PGLYRP2 is down regulated when exposed to cartilage Interleukin-1 beta (IL-1β) treatment and cartilage injury. He observed that although IL-1β usually increases PGLYRP2 levels, mechanical injury could enhance chondrocyte exposure to cytokines, influencing this response.

Additionally, Saha et al[9] demonstrated that PGLYRP2 is essential for the development of peptidoglycan or Muramyl dipeptide (MDP) induced local inflammation and arthritis. They found that PGLYRP2 deficient mice, along with Nod2 and TLR4 deficient mice were all resistant to arthritis induced by peptidoglycan or MDP and it is known that musculoskeletal involvement and arthritis is one of the most common manifestations of SLE.

There were statistically non-significant correlations between PGLYRP2 fold increase and age, disease duration, number of affected joints, hemoglobin, TLC, Platelet Count, ESR, ALT, AST, urea, creatinine, uric acid, urine protein, Na, K, serum Ca and albumin. These findings may be due to differences in individual patient characteristics and the heterogeneity of SLE.

Our study revealed a statistically significant difference between SLE patients and the control group regarding serum PGLYRP2 levels as it were significantly higher in cases with SLE with range between (1.34-5.73) ng

/mL ($p< 0.05$).The cause of that is Serum PGLYRP2 which may have a scavenger function similar to amidase-active insect PGRPs, and thus may neutralize pro-inflammatory peptidoglycan, although, in tissues, PGLYRP2 has an opposite effect and participates in induction of inflammatory response[9, 20 ,21.]This result coincides with the study of Li et al [8] who found that serum PGLYRP2 was significantly higher in cases with stable SLE.

Also our results go hand in hand with Huang et al [10] who reported that PGLYRP-2 was a specific autoantigen of RA by testing anti-PGLYRP-2 levels, which were higher in RA group than that in controls. This might happen as a result of that PGLYRP2 involved in B cell activation and autoantibodies production in patients of RA

There was a statistically significant correlation betweenC3, C4 and serum PGLYRP2 levels by ELISA ($p<0.05$). This result coincides with the study of Li et al[8] who revealed that serum level of PGLYRP2 was significantly correlated to C3,C4 in SLE patients. Our findings could be attributed to differences in the severity of SLE among participants might influence serum levels of C3 and PGLYRP2 as the decrease in complement levels indicates its involvement in antibody defense in tissues with increased disease activity [22]. Also Complement C4 is crucial for activating the classical and lectin pathways, as well as for forming C3 convertase, which is essential for generating the membrane attack complex (MAC) [23].

There was statistically non-significant correlations between 24-h urine protein and serum PGLYRP2 levels by ELISA ($p>0.05$). In contrast to our study Li et al [8]study showed that serum level of PGLYRP2 was significantly correlated to renal function parameters like 24-h urine protein in SLE patients. The reason for this disparity may be due to the timing of urine and serum sample collection relative to disease activity or flare-ups may influence results. Also different ethnic groups.

There was statistically significant correlation between SLEDAI score and serum PGLYRP2 levels by ELISA ($p<0.05$). Our results go hand in hand with Li et al [8] who found that PGLYRP2 was significantly correlated with SLEDAI.

In contrast to our study Huang et al [10], found that there was no association between anti-PGLYRP-2 level and disease activity in Rheumatoid arthritis (RA). It's likely that anti-PGLYRP-2 in RA might be play roles in the triggering stage of RA pathogenesis instead of the exacerbation stage.

Because of limited studies were done on PGLYRP2 there is another example in tumor as Yang et al [24] reported that PGLYRP2 acts as a candidate biomarker for adequate immune response against HCC and improved patient outcomes. They proved that PGLYRP2 is secreted from the liver into the blood to perform the function of immune surveillance but, parts of them are translocated from the cytoplasm into the nucleus that occurs under certain conditions as stress, such as in the cancer microenvironment, and thus regulate the immune response.

Hence, the observed increase in PGLYRP2 gene expression and serum PGLYRP2 in lupus patients may be due to two reasons. First; SLE patients had increased levels of Th17 cells, Th17/Treg ratio which produce the major cytokine IL-17 have a dominant effector and pro-inflammatory functional profile [10] , second; SLE preform stress condition so PGLYRP2 is secreted into the blood from the liver.

CONCLUSION

Our results support the hypothesis that PGLYRP2 contributes in the development of the pathogenesis of SLE, disease activity. PGLYRP2 appears to be a useful marker with good sensitivity. Measuring PGLYRP2 level can aid in guide the early management of SLE.

Conflicts of interest:

- The authors declare that they have no financial or non-financial conflicts of interest related to the work done in the manuscript.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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