ORIGINAL ARTICLE

Association of serum PYCARD and NLRP3 inflammasome gene polymorphism with rheumatoid arthritis and disease activity

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ABSTRACT

Background: *Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by joint inflammation, cartilage destruction, and disability. Activation of the nucleotide-binding leucine-rich repeat receptor (NLR) family pyrin domain- containing 3 (NLRP3) inflammasome, and its adaptor protein PYCARD (ASC) plays a crucial role in pathogenesis by promoting the release of interleukin-1β and IL-18, and pyroptotic cell death. Genetic variations such as the NLRP3 rs4612666 single-nucleotide polymorphism (SNP) may influence the activity and susceptibility to RA. Understanding the interplay between serum PYCARD levels and NLRP3 gene polymorphisms could provide insights into RA prognosis.* ***Aim:*** *This study aimed to investigate the association between serum PYCARD levels and NLRP3 rs4612666 polymorphism with RA susceptibility and disease activity.* ***Methodology:*** *This comparative cross-sectional study included 21 RA patients and 21 age- and sex-matched healthy controls. Serum PYCARD levels were quantified using Enzyme-Linked Immunosorbent Assay. Genotyping of the NLRP3 rs4612666 polymorphism was performed using Polymerase Chain Reaction and restriction fragment length polymorphism analysis.* ***Results:*** *Serum PYCARD levels were significantly higher in RA patients compared to controls and positively correlated with disease activity and inflammatory markers. The NLRP3 rs4612666 CC genotype was associated with increased RA susceptibility and higher disease activity scores, while the TT genotype appeared protective. Multivariate analysis revealed that serum PYCARD and the CC genotype are potential predictors of RA and its activity.* ***Conclusion:*** *Elevated serum PYCARD and NLRP3 rs4612666 SNP are strongly associated with RA susceptibility and disease activity. These markers may serve as tools for early diagnosis, prognosis, and personalized therapy in RA.*

INTRODUCTION

Rheumatoid arthritis is the most common chronic autoimmune inflammatory joint disease and represents the primary cause of disability, comorbidities, and mortality worldwide. Clinically, it is defined by symmetric joint swelling, pain, morning stiffness, and progressive destruction of synovial joints[1].

The etiology of RA is multifactorial, involving the interplay between genetic predisposition and environmental triggers. Central to its pathogenesis is the dysregulation of both adaptive and innate immune responses, leading to persistent synovial inflammation, pannus formation, and cartilage and bone destruction. Increasing evidence has highlighted the role of the NLRP3 inflammasome, a cytosolic multiprotein complex, in bridging innate and adaptive immunity and amplifying inflammatory cascades in RA [2].

The NLRP3 inflammasome comprises NLRP3 as the sensor, pro-caspase-1 as the effector, and the adaptor protein PYCARD (ASC)[3]. Upon activation, PYCARD facilitates the recruitment of pro-caspase-1, resulting in caspase-1 activation and subsequent release of proinflammatory cytokines like IL-18 and IL-1β, in addition to pyroptotic cell death induction. Aberrant activation of this pathway contributes to chronic synovial inflammation and joint damage [4].

Furthermore, genetic variations have been shown to influence RA susceptibility and progression. In particular, the NLRP3 gene polymorphism rs4612666 has been associated with increased disease risk and activity. Such SNPs may alter inflammasome regulation, thereby sustaining chronic inflammation and offering potential targets for predictive biomarkers and personalized therapeutic strategies [5]. Thereby, the current study aimed to examine the association between serum PYCARD and the NLRP3 gene rs4612666 SNPs with RA and disease activity.

METHODOLOGY

This comparative cross-sectional study included 42 participants recruited from the Rheumatology, Physical Medicine and Rehabilitation Department, Benha University Hospital, after obtaining informed consent. The work was conducted at the Microbiology and Immunology Department, Benha Faculty of Medicine, between December 2024 and March 2025. The present study protocol was approved by Benha University Research Ethics Committee (Approval code: MS 17-6-2024). Before being enrolled in the study, each participant provided a written informed consent.

Subjects:

Group 1 (RA): 21 patients ≥18 years fulfilling American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) classification criteria for RA published in 2010 [6]. Exclusions: other autoimmune, metabolic, cardiovascular, renal, hepatic diseases, pregnancy or lactation.

Group 2 (controls): 21 age- and sex-matched healthy individuals without relevant diseases, pregnancy or lactation.

Patients were evaluated through a complete medical history taking and clinical examination. The RA activity was assessed using the disease activity score (DAS-28)[7]. The patients' functional status was evaluated using the Health Assessment Questionnaire Disability Index (HAQ-DI). Calculation of HAQ-DI involves answering a questionnaire that includes questions under eight domains: dressing, rising, eating, walking, hygiene, grip, reach, and usual activities[8]. Patients were requested to indicate their global assessment of pain on the visual analogue scale (VAS), which ranged from 0 to 10 cm[9]. Other laboratory data, as anti-Cyclic Citrullinated Peptide antibodies (Anti-CCP), rheumatoid factor (RF), C-Reactive Protein (CRP) and Erythrocyte Sedimentation Rate (ESR), were obtained from patients' medical records.

Sampling: From each participant, 6 mL of whole blood was collected. 2 mL were put in EDTA tubes and stored at −80°C for PCR, while 4 mL were collected without anticoagulant, allowed to clot, and centrifuged at 4000 r/min for 15 min. About 500 µL of serum was separated and stored at −80°C for ELISA.

Detection of Human PYCARD (ASC) by ELISA:

Serum PYCARD levels were measured using a commercial Sandwich-ELISA kit, Human PYCARD (PYD and CARD Domain Containing Protein *(Bioassay Technology Laboratory ELISA kit; Shanghai Korain Biotech Co., Ltd., Shanghai, China)* according to the manufacturer's instructions. Optical density (OD) was measured at 450 ± 2 nm using a microplate reader *(Infinite F50, Tecan Austria GmbH, Singapore; supplied by Clinilab)*. PYCARD concentrations were calculated by comparing sample OD values with the standard curve, as shown in Figure (1).

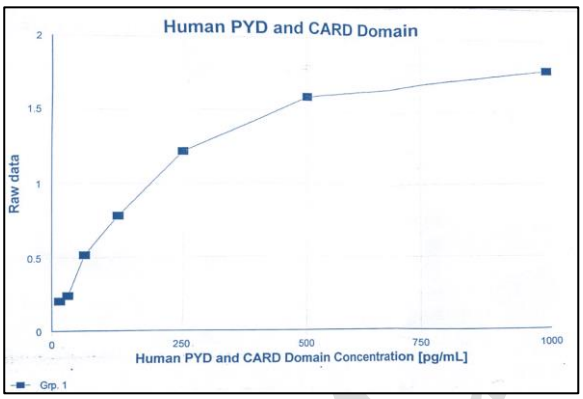

Fig. 1: Standard curve of analyzed data by ELISA

Genotyping of the NLRP3 rs4612666 SNP by PCR and RFLP analysis:

Genomic DNA was extracted from EDTA blood using QIAamp DNA Blood Mini Kit *(Qiagen, Germany)*. The NLRP3 rs4612666 SNP was assessed by PCR using specific primers (Forward: 5′-TGCTCTAAAGGATTCTGAAGG-3′, Reverse: 5′-AAGTAAGGAGGCTCTGAGAAG-3′). PCR program consisted of initial denaturation at 95°C for 5 minutes, followed by 35 cycles of 95°C for 30 s, 60°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 5 min. Amplified products were digested with restriction enzyme **BbsI** *(New England Biolabs, USA, Cat. No. R0539S)* according to the manufacturer's instructions. Two negative controls (PCR reactions without DNA template) were involved in each run to eliminate contamination. The resulting fragments were separated on 2% agarose gel stained with ethidium bromide, using Gene Ruler 100 bp DNA ladder *(Fermentas, Germany)* as a molecular size marker (bands at 100–3000 bp). Genotypes were identified based on RFLP band patterns under UV illumination.

RESULTS

This comparative cross-sectional study included 42 participants who were matched for age and sex and divided equally into two groups: 21 patients with RA satisfying the 2010 ACR/EULAR criteria and 21 apparently healthy controls. The mean age of RA patients was 50.1 ± 10.1 years versus 45.5 ± 3.5 years in controls, with an insignificant difference ($p = 0.057$). Females represented 61.9% of the RA group and 57.1% of the controls, while males accounted for 38.1% and 42.9% respectively ($p = 0.753$). Thus, both groups were well-matched demographically as shown in Figure 2 (a & b).

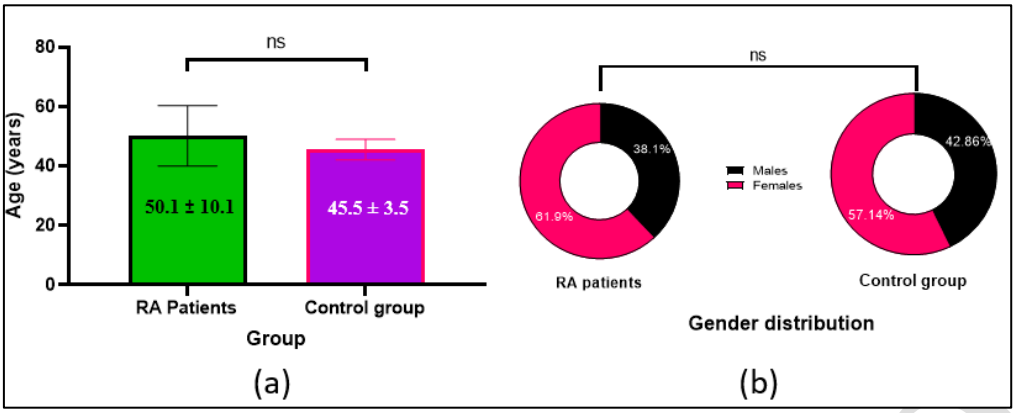

Fig. 2: (a) Age of the studied groups; (b) Sex of the studied groups

Table 1: Comparison of laboratory data between the Two Groups

		RA Patients (n=21)	Control (n=21)	p-value
CRP (mg/dL)	**Mean ± SD**	28.78± 7.91	4.40 ± 1.58	<0.001**
	Range	16.01 – 44.1	1.8 – 7.0	
ESR (mm/hr)	**Mean ± SD**	113.44 ± 19.98	15.23 ± 4.56	<0.001**
	Range	84.86 – 140.83	8.44 – 22.85	
RF (U/ml)	**Mean ± SD**	94.11 ± 40.99	12.14 ± 4.44	<0.001**
	Range	35.41 – 146.02	6.15 – 18.98	
Anti CCP (U/ml)	**Mean ± SD**	115.07 ± 31.42	10.36 ± 4.09	<0.001**
	Range	77.02 – 174.7	0.3 – 18.44	

RA: rheumatoid arthritis, CRP: C-reactive protein, ESR: Erythrocyte Sedimentation Rate, RF: Rheumatoid Factor, Anti CCP: Anti-Cyclic Citrullinated Peptide antibodies, ** = highly significant p < 0.001

RA patients showed significantly higher levels of CRP, ESR, RF, and anti-CCP compared to controls, as shown in Table (1).

Most RA patients (76.2%, n=16) had active disease, while 23.8% (n = 5) were inactive, as shown in Figure (3).

PYCARD was significantly higher in RA patients; each unit increase raised RA risk by 76% (p < 0.001), as observed in Table (2).

Serum PYCARD showed a significant association with clinical parameters, showing moderate positive correlations with DAS-28 (r = 0.500, p = 0.002) and VAS (r = 0.488, p = 0.004), and a weak correlation with HAQ-DI (r = 0.338, p = 0.040) in RA patients, as shown in Figure (4 a, b, c)

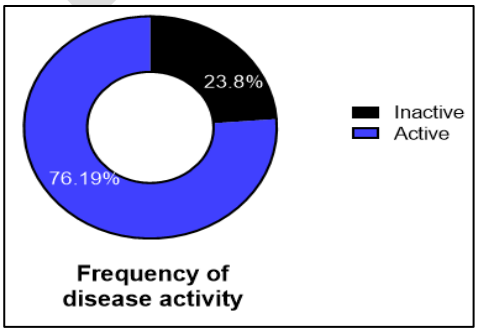

Fig. 3: Frequency of disease activity of the RA patients

Table 2: Serum PYCARD concentration among the studied groups

		Bivariate analysis			Regression analysis	
		RA patients (n=21)	Controls (n=21)	p-value	OR (95% CI)	p-value
PYCARD (pg/mL)	Median IQR	1768.10 1195.10-1826.10	1053.0 750.05 - 1083.57	<0.001**	1.76(1.27-2.44)	<0.001**

RA: rheumatoid arthritis, CI=Confidence interval, OR=Odds ratio, **: Highly significant as p value < 0.001

Table 3: Association between disease activity and serum PYCARD in RA patients

		Bivariate analysis			Regression analysis	
		Inactive (n=5)	**Active (n=16)**	**p-value**	**OR (CI95%)**	**p-value**
PYCARD (pg/mL)	**Median**	975.15	1790.00	0.003**	1.02 (1.01-1.03)	0.010*
	IQR	498.75-1416.32	1728.03-1843.38			

OR=Odds ratio, RA: rheumatoid arthritis, CI=Confidence interval, **highly significant P < 0.001, *Significant p < 0.05

Serum PYCARD levels were significantly higher in active RA, and elevated levels increased the odds of active disease (OR = 1.02, p = 0.003), as shown in Table (3).

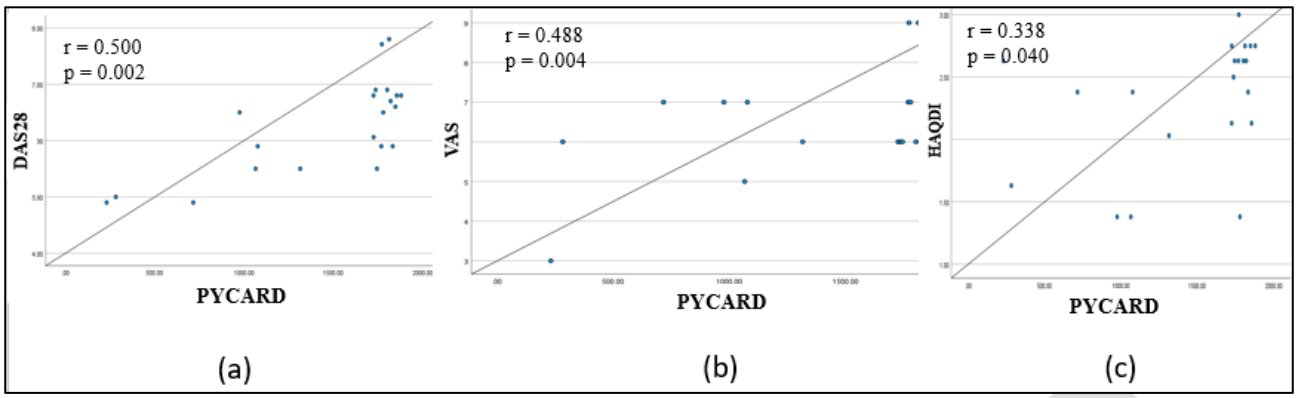

Fig. 4: Spearman's correlation between serum PYCARD concentration and DAS-28, VAS, and HAQ-DI scores among RA patients

Table 4: Association between NLRP3 rs4612666 genotypes and the susceptibility to RA

		Bivariate analysis			Regression analysis	
		RA patients (n=21)	Controls (n=21)	p-value	OR (95% CI)	p-value
Multiplicative model						
TT	n (%)	2 (9.5%)	12 (57.1%)	<0.001**	Ref.	<0.001**
TC	n (%)	6 (28.5%)	8 (38.2%)		4.5 (0.72 – 28.1)	
CC	n (%)	13 (62%)	1 (4.7%)		32.5 (6.25 – 979.4)	
HWE		✔ Yes	✔ Yes			
χ^2		0.302	0.051			
p-value		0.58	0.82			
Dominant model						
TT	n	2	12	<0.001**	Ref	Ref
(TC + CC)	n	19	9		12.67 (2.23-68.93)	0.003**
Recessive models						
CC	n	13	1	<0.001**	Ref	Ref
(TT + TC)	n	8	20		0.03 (0.00-0.28)	0.002**
Alleles						
T	n (%)	10 (23.8)	32 (76.2)	<0.001**	Ref	Ref
C	n (%)	32 (76.2)	10 (23.8)		10.24 (3.75-27.95)	<0.001**

RA: rheumatoid arthritis, OR = Odds Ratio, N= number, Ref. = Reference, CI = Confidence Interval, ** = highly significant p < 0.001, HWE: Hardy–Weinberg Equilibrium, χ^2: Chi-square value for goodness-of-fit.

There was a highly significant association between NLRP3 rs4612666 and RA susceptibility. The CC genotype and C allele were strongly linked to increased risk, supported by dominant and recessive models, with all groups in Hardy–Weinberg equilibrium as found in Table (4).

There was a significant association between NLRP3 rs4612666 and RA activity. C allele and CC genotype were strongly linked to higher odds of active disease, confirmed by both recessive and dominant models ($p < 0.05$), as shown in Table (5).

Serum PYCARD levels differed significantly across NLRP3 rs4612666 genotypes in RA patients, with the CC genotype linked to higher levels (OR = 1.48, p = 0.015). RA patients carrying the C allele also had higher median PYCARD than those with the T allele (1778.9 vs 1064.5; OR = 2.54). Similarly, controls with the C allele showed higher PYCARD levels than those with the T allele (1056.9 vs 563.5; OR = 3.54), as shown in Table (6).

Patients with CC had a higher mean HAQ-D1 (2.41) than TT (1.63) and TC (1.70), while no significant differences were seen in DAS-28 and VAS scores, as shown in Figure (6).

Univariate analysis showed that elevated CRP, ESR, RF, anti-CCP, DAS-28, VAS, HAQ-DI, PYCARD, and the CC genotype were linked to higher RA risk. Multivariate analysis confirmed that the CC genotype, along with high CRP and PYCARD, independently increased RA risk, as shown in Table 7.

Table 5: The association between NLRP3 rs4612666 genotypes and RA activity

		Bivariate analysis			Regression analysis	
		Active disease n=16 n (%)	Inactive disease n=5 n (%)	p-value	OR (CI 95%)	p-value
Multiplicative model						
TT	n (%)	0 (0.0)	2 (40.0)	<0.001**	Ref	Ref
TC	n (%)	4 (25.0)	2 (40.0)		1.04 (1.01-1.23)	0.013*
CC	n (%)	12 (75.0)	1 (20.0)		32.45 (7.89-43.65)	0.010*
Dominant model						
TT	n (%)	0 (0.0)	2 (40.0)	<0.001**	Ref	Ref
(TC + CC)	n (%)	16 (100.0)	2 (60.0)		9.54 (1.24-11.98)	0.41*
Recessive models						
CC	n (%)	12 (75.0)	1 (20.0)	<0.001**	Ref	Ref
(TT + TC)	n (%)	4 (25.0)	4 (80.0)		0.59 (0.23-0.67)	0.011*
Allele type						
T allele	n (%)	4 (12.5)	6 (60.0)	0.002**	Ref	Ref
C allele	n (%)	28 (87.5)	4 (40.0)		10.5 (2.03-54.27)	0.005**

RA: rheumatoid arthritis, CI=Confidence interval, OR=Odds ratio, ** = highly significant p < 0.001, *Significant p < 0.05

Table 6: NLRP3 rs4612666 genotypes and serum PYCARD levels in RA patients and controls

	RA patients					Control group				
	Bivariate analysis			Regression analysis		Bivariate analysis			Regression analysis	
	PYCARD (pg/mL)		p-value	OR (CI95%)	p-value	PYCARD (pg/mL)		p-value	OR (CI95%)	p-value
	Median	IQR				Median	IQR			
TT	673.14	281.74-673.14	<0.001**	0.45 (0.27-0.75)	0.004**	141.40	141.40-141.40	<0.001**	0.13 (0.06-0.27)	<0.001**
TC	1195.10	910.30-1763.25		1.02 (1.01-3.47)	0.013	750.05	437.87-993.79		0.56 (0.41-0.78)	0.002**
CC	1801.00	1755.90-1839.55		1.48 (1.09-2.01)	0.015*	1073.40	1053.35-1165.78		1.24 (1.14-2.36)	0.001**
T	1064.54	607.25-1416.75	<0.001**	0.45 (0.33-0.78)	<0.001**	563.51	207.9-921.22	<0.001**	0.22 (0.10-0.44)	<0.001**
C	1778.9	1736.5-1831.9		2.54 (1.39-7.89-	<0.001**	1056.9	1032.9-1090.34		3.54 (1.69-8.77)	<0.001**

RA: rheumatoid arthritis, *Significant p < 0.05 ** = highly significant P < 0.001, OR: Odds Ratio, CI95%: Confidence Interval

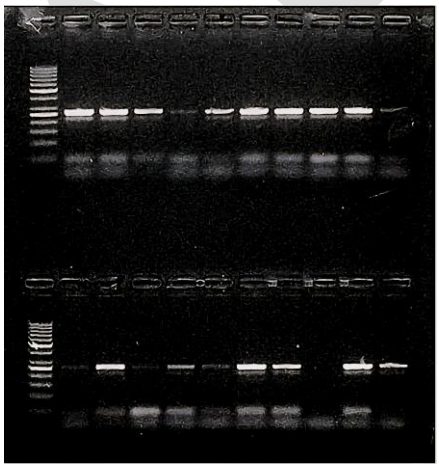

Fig. 5: Agarose gel electrophoresis showing PCR-RFLP analysis of NLRP3 rs4612666 polymorphism

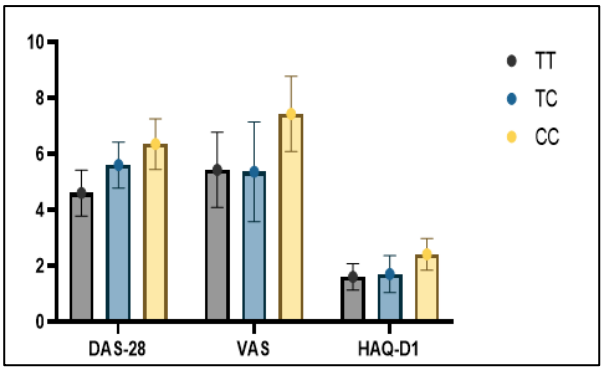

Fig. 6: Association of NLRP3 rs4612666 genotypes with DAS-28, VAS, and HAQ-DI scores in RA patients.

Table 7: Predictors of RA

		Univariate analysis		Multivariate analysis	
		OR **(95%CI)**	P value (univariate)	OR **(95%CI)**	P value (multivariate)
Age		1.09	0.061		
Sex	Males	Ref			
	Females	1.219 (0.36-4.19)	0.753		
CRP (mg/dL)		1.13 (1.10-11.04)	0.023*	1.26 (1.1-10.6)	0.035*
ESR (mm/hr)		1.03 (1.01-1.05)	0.004**	1.14 (0.95-1.36)	0.458
RF		1.03 (1.01-1.05)	0.012*	1.36 (0.87-3.69)	0.369
Anti CCP		1.03	0.002**	1.02 (0.98-2.39)	0.444
DAS 28		10.41 (2.63-41.17)	<0.001**	1.36 (0.47-3.65)	0.413
VAS		2.66 (1.38-5.13)	0.004**	2.0 (0.461-3.26)	0.221
HAQ-D1		13.85 (3.09-62.11)	<0.001**	1.86 (0.14-1.98)	0.136
NLRP3 polymorphism	TT	Ref		Ref	
	TC	4.5 (0.72-28.15)	0.108	2.65 (0.68-30.45)	0.310
	CC	78.0 (6.24-974.71)	<0.001**	80.01 (12.69-101.65)	0.001**
Serum PYCARD (pg/mL)		1.02 (1.01-1.04)	0.006**	3.12 (2.65-6.87)	0.005**

RA: rheumatoid arthritis, DAS: Disease Activity Score, VAS: Visual Analog Scale, HAQ-D1: Health Assessment Questionnaire - Disability Index, OR=Odds ratio, CI=Confidence interval, RF=Reference category, * statistically significant P value ≤ 0.05, ** = highly significant P value < 0.001

Table 8: Predictors of disease activity in RA patients

		Univariate analysis		Multivariate analysis	
		OR (95%CI)	P value (univariate)	OR (95%CI)	P value (multivariate)
Age		1.05 (0.94-1.17)	0.368		
Sex (Ref=Males)	Males	Ref		Ref	
	Females	3.30 (0.41-26.37)	0.260		
CRP (mg/dL)		0.93 (0.83-1.03)	0.165		
ESR (mm/hr)		0.98 (0.95-1.0)	0.107		
RF		0.99 (0.97-1.02)	0.594		
Anti CCP		0.99 (0.97-1.02)	0.770		
DAS 28		1.85 (0.50-6.84)	0.354		
VAS		2.82	0.130		
HAQ-D1		17.10 (1.40-209.38)	0.026*	12.36 (0.98-18.92)	0.145
NLRP3 polymorphism	TT	Ref		Ref	
	TC	3.64 (2.57-3.97)	0.034*	2.45 (1.36-4.67)	0.001**
	CC	6.69 (2.59-10.36)	0.013*	7.98 (5.69-10.36)	0.002**
Serum PYCARD (pg/mL)		1.02 (1.01-1.03)	0.010*	3.06 (1.02-6.78)	0.001**

RA: rheumatoid arthritis, VAS: Visual Analog Scale, DAS: Disease Activity Score, HAQ-D1: Health Assessment Questionnaire - Disability Index, OR=Odds ratio, Ref=Reference category, CI=Confidence interval و * statistically significant P value ≤ 0.05

Higher HAQ-DI and serum PYCARD levels were linked to increased risk of active RA, while multivariate analysis showed that TC/CC genotypes with high PYCARD further raised this risk, as shown in Table 8.

DISCUSSION

Normal genetic variations, especially SNPs, are known to influence immune regulation in RA. Variants within inflammasome-related genes such as NLRP3 may alter PYCARD activation and inflammatory mediator release, thereby affecting individual susceptibility, disease activity, and clinical outcomes[10].

In our study, RA patients and controls showed no significant differences in age or sex, indicating well-matched groups. This finding aligns with those of Ahmed and Zaki[11], Alharthi et al.[12], and Ramírez et al.[13], who also reported no demographic differences between RA and control groups.

Regarding laboratory data reported in our study, RA patients had significantly higher RF, ESR, CRP, and anti-CCP than controls. This was consistent with the findings of Elghouneimy et al.[14], Alhussain et al.[15], and Balaji et al.[16]. In contrast, Choi et al.[17] reported the presence of these antibodies in some healthy individuals, most likely due to population, age, sex, or study design differences, indicating that these biomarkers are useful but not fully specific for RA.

The majority of RA patients involved in our study had moderate to high disease activity as shown by DAS-28 score, reflecting a high inflammatory burden. Similar findings were reported in other studies [18,19,20]. Notably, this was inconsistent with Al-Saleh et al.[21] and Tang et al.[22], who observed higher remission rates among RA patients that can be attributed to earlier diagnosis, strict treatment strategies, and wider access to biologic therapies.

Our study showed significantly elevated serum PYCARD in RA patients compared with healthy controls, supporting its role in RA pathogenesis. Similar findings were informed by Messelink et al.[23]. In contrast, Ricci et al.[24] and Lee et al.[25] didn't find significant differences, which may be due to disease heterogeneity or ethnic variations.

Serum PYCARD levels were significantly increased in cases with active disease than those in remission and correlated positively with DAS-28 score. In this regard, our results agree with Alotaibi et al.[26] and Zhang et al.[27]. However, Ricci et al.[24] and Lee et al.[25] didn't observe such correlations, possibly due to sample size differences and population-specific factors.

Furthermore, our study demonstrated a significant association between NLRP3 rs4612666 polymorphism and RA susceptibility, highlighting the role of innate immunity and inflammasomes in disease pathogenesis. Similar associations were published in different studies [28,29,30]. In contrast, other authors[31,32] reported no association, and they further explained this finding by the differences in sample size, population genetics, or environmental factors.

Additionally, our study revealed that the C allele and CC genotype of NLRP3 rs4612666 were linked to higher RA activity, while TT was more frequent in remission, suggesting a role in both susceptibility and disease activity. Similar associations were reported by Al-Khodair et al.[33]and Huang et al.[34]. In contrast, Wang et al.[35] found no link, most likely due to ethnic, sample size, or treatment-related differences.

Our results showed that RA patients with the C allele and the CC genotype of rs4612666 had significantly higher serum PYCARD levels than TT genotype and controls, suggesting a role in inflammasome activation and systemic inflammation. This is supported by Huang et al.[34], who linked the C allele to increased IL-1β and PYCARD activation. In contrast, Kadam et al.[31] and Uğurlu et al.[32] found no association, most likely due to ethnic genetic variability and treatment effects that may mask genotype-related differences.

Moreover, the C allele and CC genotype of NLRP3 rs4612666 were linked to higher DAS-28, VAS, and HAQ-DI, suggesting a role in modulating disease activity and severity. Similar associations were described by Al-Khodair et al.[33] and Huang et al.[34]. In contrast, Choulaki et al.[36] found no association, most likely due to population-specific genetic differences.

Significantly high serum PYCARD levels were identified among RA patients compared to the control. Furthermore, a positive correlation between serum PYCARD levels and DAS-28, VAS, and HAQ-DI was demonstrated, indicating a link between inflammasome activation and inflammation, pain, and disability in RA. Similar results were reported by Ibrahem et al.[37]and Jiang et al.[38]. In contrast, Demir et al.[39] found no such association, likely due to differences in disease characteristics or treatment effects.

Our study showed that high CRP, ESR, RF, anti-CCP, PYCARD, and the CC genotype increased the risk of developing RA, with CC plus high CRP and PYCARD being the strongest predictors. This agrees with Awni et al.[2], while Wang et al.[30] did not confirm these findings, most likely due to population and design differences.

In our study, the higher HAQ-DI and serum PYCARD were linked to active RA, and multivariate analysis identified TC/CC genotypes with high PYCARD as strong disease predictors. Similar associations were reported by Awni et al.[2], and Martínez-García et al[40]. In contrast, Hassan et al.[23] found no such links, most likely due to genetic variability or treatment effects.

Some limitations of this investigation ought to be recognized. First and foremost, the statistical power and generalizability of the findings may be restricted by the relatively small sample size. Second, the results may not accurately reflect the genetic and clinical diversity of other populations, as the study was conducted in a singular center. Third, only one SNP in the NLRP3 gene (rs4612666) was analyzed, whereas other polymorphisms and related inflammasome genes may also contribute to RA susceptibility and activity. Fourth, serum PYCARD levels were measured at a single time point, and longitudinal follow-up could provide more insight into their dynamic relationship with disease activity and treatment response.

Therefore, it is recommended to conduct future studies with larger, multi-center cohorts, include a broader range of SNPs and inflammasome-related genes, and apply longitudinal measurements of serum PYCARD to better clarify its role in RA pathogenesis and therapeutic outcomes.

CONCLUSION

Our study demonstrated that serum PYCARD was significantly increased in RA cases and associated with inflammatory and clinical activity markers. Elevated PYCARD levels were associated not only with greater disease activity but also with increased susceptibility to RA. The NLRP3 rs4612666 polymorphism was similarly linked to disease susceptibility and activity, with the CC genotype conferring a higher risk and the TT genotype showing a protective effect. Combined analysis further revealed that TC/CC genotypes together with high serum PYCARD levels strongly predicted active disease. Overall, these findings highlight serum PYCARD and NLRP3 rs4612666 SNP as valuable markers for RA, supporting their role in disease susceptibility, monitoring, and treatment guidance.

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