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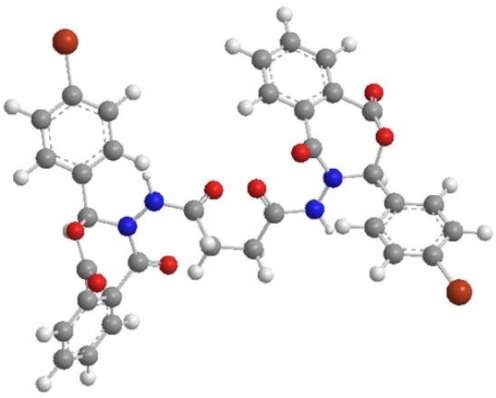

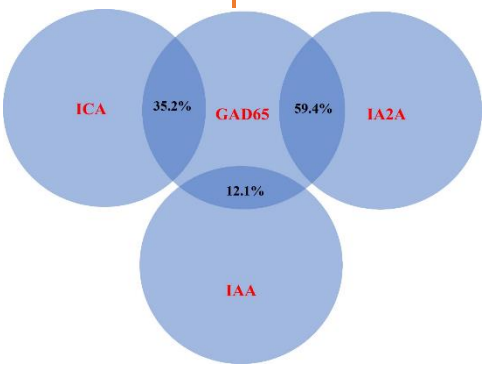

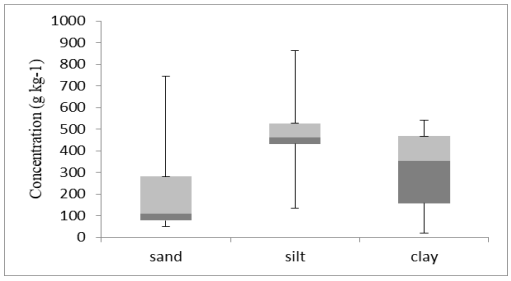

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Assessment of Wnt-5a, Anti-SMA, FGF-23, and Anti-CCP Biomarkers in Patients with Rheumatoid Arthritis

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Abstract

Background: Rheumatoid arthritis (RA) is a multifactorial autoimmune disease with unknown etiology that mainly affects synovial joints **Objectives:** To compare the level of serum biomarkers (Wnt5a, anti-SMA, FGF23, and anti-CCP) in RA patients and healthy control. **Methodology:** This case-control study was conducted on 88 patients with RA and 88 normal healthy individuals at the Rheumatology Center, Sulaimaniyah, from November 2021 to November 2022. A detailed questionnaire for the collection of sociodemographic measures was filled out for each participant. Then, the levels of Wnt5a, anti-SMA, FGF23, and anti-CCP were determined using the ELISA technique. **Results:** The patients reported higher levels of ESR and CRP than healthy controls. Most patients (63.7%) had moderate disease activity concerning DAS-28. There was a highly significant difference ($p<0.001$) between patients' serum Wnt5a, FGF23, and anti-CCP levels compared to healthy controls except for anti-SMA. Additionally, there was a significant correlation between Wnt5a and FGF23 ($p<0.001$); Wnt5a and anti-SMA ($p<0.001$); FGF23 and anti-SMA ($p<0.001$). Finally, no correlation between the DAS-28 score and biomarkers was seen in RA patients. **Conclusions:** There was no significant correlation in the patient's serum Wnt5a, FGF23, anti-SMA, and anti-CCP with different age groups, duration of RA and joint involvements.

Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory autoimmune disease mainly characterized by painful and swollen joints that significantly reduce the quality of life and physical function [1,2]. Despite a joint involvement and its heterogeneous feature [3], RA presents systemic manifestations and extra-articular symptoms, including hematologic, neurologic, renal, cardiovascular, and pulmonary disturbances with impairment of the lacrimal/salivary glands [4].

An immense amount of synovial cellularity was found in RA patients, including cellular influx, fibroblast-like synoviocytes (FLS) hyperplasia, and infiltrating immune cells' activation/proliferation/differentiation. In RA, the early stage is related to synovial vascular inflammation, a robust infiltration of leukocytes into the synovium leads to hyperplasia of the synovial lining, production of lymph follicles, and growth of the developed pannus. The more common cells in RA synovium are FLS, T-lymphocytes and monocyte-lineage cells [5].

Fibroblast growth factor-23 (FGF-23) protein contains approximately 251 amino acids (32 kDa) with a

canonical N-terminal FGF homology domain [6]. FGF23 is primarily produced and expressed in osteocytes that circulate to the kidneys and regulate bone metabolism [7]. In the advanced stages of RA, FGFR signalling plays a significant role, mainly when RA cells survive, and it is directly related to inflammation and osteoporosis in patients with RA [8].

Canonical Wnt signalling enhances the differentiation of osteoblast precursors into matured osteoblasts and increases bone formation [9]. The Wnt signalling is associated with localized and systemic bone loss in RA that is down-regulated directly or indirectly through pro-inflammatory cytokines, such as TNF-α and IL-1β (via the induction of Dkk-1 and sclerostin), IL-6, and IL-17 [10]. Smooth muscle actin (SMA) is primarily used to diagnose autoimmune hepatitis, although it is a common biomarker in RA patients [11]. According to reports, myofibroblasts that are positive for alpha-SMA are extremely important in the fibrosis and pathogenesis of RA [12].

Mainly, autoantibodies to citrullinated proteins, including filaggrin and its circular form, cyclic citrullinated peptide (CCP), are notable for their excellent sensitivity and specificity. Anti-CCP antibodies might serve as a potent serological marker for early diagnosis of RA and prognostic prediction of joint damage. Citrullinated proteins (most are fibrinous) are localized in RA synovial tissues, and anti-CCP antibodies are locally produced in RA joints [13]. According to the American College of Rheumatology, anti-CCP is a test predictive for RA; 60-70% of people with RA have anti-CCP antibodies. These antibodies are directed against CCP and might be present before the development of RA symptoms.

The primary purpose of this research was to investigate the roles and the levels of Wnt5a, SMA, and anti-CCP in the serum of RA patients. Also, to point out the correlation among these biomarkers.

Materials and Methods

Study design and setting

This case-control study was conducted on 88 RA patients and 88 healthy individuals at the Rheumatology Center, Sulaimaniyah, Iraq, from November 2021 to November 2022.

Inclusion criteria

Subjects clinically diagnosed previously to have RA for > one year by an expert clinician using confirmed laboratory tests such as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and rheumatoid factor (RF) aged between 20 to 70 years were involved in this study.

Exclusion criteria

Patients with chronic diseases (diabetes, hypertension, hyperlipidemia, heart disease, and other autoimmune diseases), breastfeeding, pregnant, and postpartum women. Also, this study were not enrolled with those patients on chemotherapy, biological medications, kidney failure, and bone fracture.

Ethical consideration and patient consent

Permission has been obtained from the Ethical Committee of the College of Medicine, University of Sulaimani, Sulaimaniyah, Iraq (No. 196 on September 09, 2021). Also, approval was taken from the Sulaimaniyah Directorate of Health, Ministry of Health. On the other hand, written informed consent was taken from the patients.

Questionnaire

A questionnaire for collecting participants' information (age, sex, marital status, residency, and occupation) and anthropometric (weight and height to determine body mass index; BMI) measures were filled out for each participant. Additionally, the subjects were asked about the age at which RA has been diagnosed, genetic history of RA/other chronic diseases, history of hospital admission due to the severity of RA, and RA complications.

Collection of blood samples

About 7.0 mL of venous blood was collected from each participant. Then, 2 ml of blood was collected in an EDTA tube to perform ESR and the other 5 mL was placed in a clot activator gel tube, allowed to clot at room temperature, then centrifuged at 6000 RPM/10 min, and sera were separated. The sera were dispensed into 3 sterile Eppendorf tubes, and kept in a freezer (-80°C) until use.

Measurement of immunological parameters using the ELISA technique

The ELISA kit (Aeskulisa, Diagnostic GmbH, Wendelsheim, Germany) was used to detect IgG antibodies specific to CCP by indirect ELISA technique. In contrast, (ELK Biotechnology, China) were used to determine the concentrations of FGF-23 and Wnt5a by sandwich ELISA technique, On the other hand, (BT Laboratories, China) was used to determine ASMA concentration quantitatively by sandwich ELISA technique. Then, (Aeskulisa, Diagnostic GmbH, Wendelsheim, Germany) was used to detect RF-check IgG, IgM and IgA in human serum using indirect ELISA technique. The samples were read by ELISA reader (Chromate awareness technology, USA) absorbance of the samples were read at 450 nm. The results were calculated by drawing a standard curve and plotting the markers concentration of the standards on the horizontal axis and the corresponding absorbance on the vertical axis.

Statistical analysis

The data were analyzed using Statistical Package for the Social Sciences (SPSS, version 26). Shapiro-Wilk test and Kolmogorov–Smirnov test were used to determine the normal distribution of the data. Mann-Whitney U and Kruskal Wallis tests were used to test the non-normally distributed quantitative variables. ANOVA test was used for normally distributed quantitative variables, while the chi-square test was used to test the categorical data. Spearman (Rho) correlation coefficient was used to determine the correlation between the biomarkers. The $p \leq 0.05$ was considered statistically significant.

Results

Distribution of participants

The RA group involved 12 (13.6%) males and 76 (86.4%) females, and the disease was more predominant in females than the males, with a ratio of 6.33:1. The mean age of RA patients was 49.42±9.46, while the mean BMI was 26.58±3.54 in males and 28.78±4.71 in females. The healthy controls comprised 22 (25%) males and 66 (75%) females, whose mean age was 43.77±10.39, mean BMI was 27.19±3.44 and 27.49±3.40 in males and females, respectively. There was a significant age difference ($p<0.05$), and no significant difference in gender and BMI were recorded between both groups ($p>0.05$) (Table 1).

Table 1: Sociodemographic characteristics of participants.

Parameter		**Participant**			**p-value**
		Patient (*n*=88)	**Control (*n*=88)**	**Total (*n* =176)**	
Gender	Male	12 (13.6%)	22 (25%)	34 (19.3%)	0.056
	Female	76 (86.4%)	66 (75%)	142 (80.7%)	
Age (Mean±SD)		49.42 ± 9.46	43.77 ± 10.39	46.6 ± 10.3	0.00*
BMI (Mean±SD)	Male	26.58 ± 3.54	27.19 ± 3.44	26.88 ± 3.49	0.083
	Female	28.78 ± 4.71	27.49 ± 3.40	28.13 ± 4.05	

BMI= body mass index; *=Significant difference using chi-square (gender) and Mann–Whitney U tests (age and BMI)

RF, CCP and genetic history of RA in participants

All healthy controls were seronegative for RF and anti-CCP, while 62 (75%) were RF seropositive in the patient group. Regarding anti-CCP, 42 (47.7%) were positive, and 46 (52.3%) were negative, with significant differences between both groups in the RF and anti-CCP. A highly significant difference was documented

between the two groups regarding the genetic history of RA, and 5.7% of the healthy controls had a genetic history of RA. At the same time, 39 (44.3%) patients had a genetic history of RA. Neither group recorded a significant difference in smoking sensation (Table 2).

Table 2: RF, CCP, genetic history of RA and smoking in the patients and controls.

Parameter		Participant		p-value
		Patient	Control	
		Number (%)		
RF	Positive	62 (75)	0 (0.0)	<0.001**
	Negative	26 (25)	88 (100)	
CCP	Positive	42 (47.7)	0 (0.0)	<0.001**
	Negative	46 (52.3)	88 (100)	
Genetic history	No	49 (55.7)	83 (94.3)	<0.001**
	Yes	39 (44.3)	5 (5.7)	
Smoking	Non- smoker	78 (88.6)	71 (80.7)	0.143
	Smoker	10 (11.4)	17 (19.3)	
Total		**88 (100)**	**88 (100)**	

CCP: cyclic citrullinated peptide; RF: Rheumatoid factor; ******=Highly significant difference using Pearson's chi-squared test

Distribution of disease duration and DAS-28 of RA patients

Regarding the disease duration, 63 (71%) of the patients had the disease for >36 months, 12 (13.6%) were between 12-36 months, and 13 (14.8%) had the RA for < 1 year. In respect of DAS 28, 56 (63.7%) patients had moderate disease activity, followed by high disease activity (26.1%), then low disease activity (6.8%). Disease remission recorded the lowest value in patients (3.4%) (Table 3).

Table 3: Frequency of disease duration and DAS-28 of RA patients.

Parameter		Number (%)
RA duration Mean±SD (83±81.32)	<12 Months	13 (14.8)
	12 -36 Months	12 (13.6)
	<36 Months	63 (71.6)
DAS-28 M±SD (4.41±1.02)	Disease remission	3.0 (3.4)
	Low disease activity	6.0 (6.8)
	Moderate disease activity	56 (63.7)
	High disease activity	23 (26.1)
Total		88 (100)

RA=Rheumatoid arthritis; DAS=disease activity score

Anti-CCP and RF cross-tabulation of RA patients

The total number of RA patients who were positive for anti-CCP was 42 (47.73%), and 46 (52.27%) were negative, while those who were positive for RF were 62 (70.46%) patients and 26 (29.54%) were negative. In all RF-positive patients, 37 (42.05%) were positive for anti-CCP tests, and 25 (28.41%) were negative. In addition, the anti-CCP positive in RF adverse reaction was only 5 (5.68%), while 21 (23.86%) were negative for anti-CCP antibody (p=0.001) (Table 4).

Table 4: Anti-CCP and RF cross-tabulation of RA patients.

RF		Anti-CCP		Total	P-value
		Negative	Positive		
		Number, %			
RF	**Negative RF**	21 (23.86)	5 (5.68)	26 (29.54)	0.001**
	Positive RF	25 (28.41)	37 (42.05)	62 (70.46)	
Number, %		46 (52.27)	42 (47.73)	88 (100)	

RF: Rheumatoid factor; CCP: cyclic citrullinated peptide; ******: Highly significant difference (p<0.001) using Pearson's chi-squared test.

ESR and CRP estimation

In respect of both ESR and CRP, the highest mean level was noted in patients (29.61±21.93 and 10.53±11.32) compared to the negative control group (8.41±4.33 and 2.76±1.61), respectively, with highly significant difference between them (p<0.001) (Table 5).

Table 5: ESR and serum level of CRP in patients and healthy controls.

Parameter Mean±SD	Participant		p-value
	Patient (*n*=88)	Control (*n*=88)	
ESR	29.61 ± 21.93	8.41 ± 4.33	<0.001**
CRP	10.53 ± 11.32	2.76 ± 1.61	<0.001**

ESR= Erythrocyte sedimentation rate; CRP= C-reactive protein; **=Highly significant difference using Mann–Whitney U test.

Rheumatoid arthritis biomarkers in the participants

The highest mean serum level of all the biomarkers (Wnt5a, FGF2, anti-SMA, and anti-CCP) were recorded in the patient's group rather than the controls. The mean of Wnt5a was 5.27±4.11 in patients and 3.61±1.83 in control (p=0.039), while the mean of FGF2 was 228.81±159.91 in the patients and 168.29±82.5 in the controls (p=0.033). Regarding anti-SMA, the mean serum level was 45.01±49.42 in the patients and 34.36±6.19 in the healthy individuals, without significant difference. Finally, a highly significant difference was noted in the serum concentration of anti-CCP (p<0.001); the mean serum level was 123.84±143.4 in the patients and 2.83±1.86 in the control group (Table 6).

Table 6: Comparing the biomarkers (Wnt5a, FGF2, anti SMA and anti-CCP) between groups.

Biomarker (M ± SD)	Participant		p-value
	Patient (*n*=88)	Control (*n*=88)	
Wnt5a (ng/mL)	5.27±4.11	3.61±1.83	0.039*
FGF23 (pg/mL)	228.81±159.91	168.29±82.5	0.033*
Anti- SMA (ng/mL)	45.01±49.42	34.36±6.19	0.477 ns
Anti-CCP (ng/mL)	123.84±143.4	2.83±1.86	< 0.001**

*: Significant difference; **: Highly significant difference using the Mann–Whitney U test

Correlation of age, symptoms, and duration of RA with the biomarkers of the patients

There was no significant correlation (p≥0.05) between biomarkers and age, duration of RA, and joint involvements (Table 7).

Table 7: Correlation of the patient's age, symptoms, and duration of RA with the biomarkers.

Variable		Biomarkers			
		Wnt5a	FGF23	Anti-SMA	Anti-CCP
		Mean±SD			
Age (Years)	**< 40**	4.92±1.69	197.46±34.50	35.53±5.15	2.99±1.71
	40-50	5.77±5.38	240.38±189.69	34.83±5.90	2.91±1.60
	> 50	4.84±2.95	225.28±147.91	32.67±7.21	2.59±2.24
	P-value	0.962 ns	0.728 ns	0.468 ns	0.833 ns
	Spearman (*Rho*)	-0.037	0.056	-0.062	-0.021
Duration of RA	**< 12 Months**	4.7±1.13	187.52±25.95	52.04±69.37	156.73±156.14
	12-36 Months	5.14±2.23	178.19±72.46	35.53±11.55	167.61±151.46
	>36 Months	5.40±4.75	246.92±183.4	45.37±49.44	108.71±138.73
	P-value	0.548 ns	0.082 ns	0.958 ns	0.475 ns
	Spearman (*Rho*)	-0.078	0.169	-0.026	-0.124
Joint involvement	**1 Large Joint**	6.23±4.77	309.51±244.25	65.90±78.55	87.54±147.04
	2-10 Large Joints	4.38±3.34	215.62±97.61	37.43±27.17	118.78±152.82

	1- 3 Small Joints	4.78±1.63	204.24±43.46	71.45±93.33	87.35±146.37
	4-10 Small Joints	5.22±3.48	206.76±76.49	34.83±8.45	136.07±137.24
	> 10 Joints	6.85±5.27	295.35±330.64	65.16±87.03	129.28±157.45
	P-value	0.709 ^{ns}	0.969 ^{ns}	0.658 ^{ns}	0.529 ^{ns}
	Spearman (*Rho*)	-0.038	0.023	-0.098	0.024

RA= Rheumatoid arthritis*;* ns=No Significant difference using Performed by Kruskal–Wallis and Spearman correlation tests

Correlation of symptoms and duration of RA to the age of the patients

There was no significant correlation in the age of the patients between symptoms (joint involvement) as well as the duration of RA patients (p>0.05) (Table 8).

Table 8: Correlation of symptoms and duration of RA to the age of the patients.

Variable		**Age of the patients (Mean ± SD)**	**P-value**
Joint involvement	1 Large Joint	56 ± 11.66	0.239 ^{ns}
	2-10 Large Joints	48.96 ± 8.28	
	1- 3 Small Joints	43.43 ± 7.09	
	4-10 Small Joints	49.49 ± 9.28	
	> 10 Joints	50.64 ± 11.33	
Duration of RA (Months)	< 12 Months	49.38 ± 12.63	0.655 ^{ns}
	12-36 Months	51.75 ± 8.23	
	>36 Months	48.98 ± 9.02	

ns=No Significant difference using one-way ANOVA test

Correlation of DAS28 score to the duration of RA of the patients

A significant positive correlation was observed between the DAS28 score and the time of RA (Rho=0.3, p=0.008) (Figure 1).

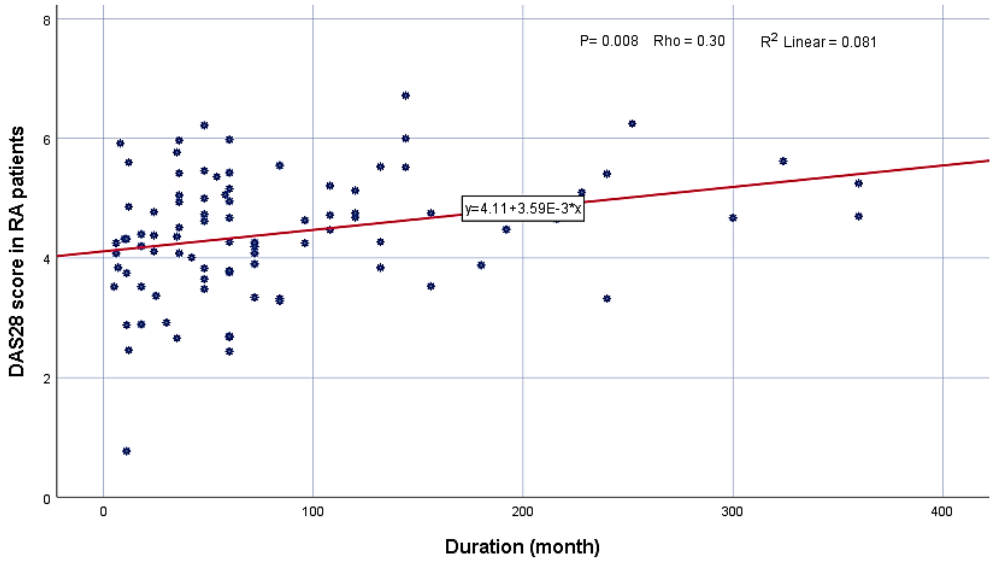

Figure 1: Correlation between DAS-28 score and Duration of RA.

Correlation between DAS28 and biomarkers of the patients

There was no significant association (p≥0.05) between the DAS-28 score and Wnt5a, FGF-23, anti-SMA, and anti-CCP in patients with RA.

Table 9: Correlation of DAS28 with serum concentration of the biomarkers of the RA patients.

	Value	Wnt5a	FGF23	Anti-SMA	Anti-CCP
DAS-28	**p-value**	0.757 ^{ns}	0.715 ^{ns}	0.326 ^{ns}	0.664 ^{ns}
	Spearman Correlation (*Rho*)	0.023	0.04	-0.106	0.046

ns =No Significant correlation using Spearman correlation test

Correlation between the studied biomarkers in the patients

As shown in Table 10, there was a significant positive correlation between Wnt5a and FGF23 ($p<0.001$, Rho=0.892); between Wnt5a and anti-SMA ($p<0.001$, Rho=0.636); between FGF23 and anti-SMA ($p<0.001$, Rho=0.761). Lastly, there was no significant correlation between other biomarkers.

Table 10: Correlation between the biomarker in the patients.

Correlation	**Biomarkers**		
	Wnt5a	**FGF23**	**Anti-SMA**
FGF23	$p<0.001$ ** *Rho* = 0.892		
Anti-SMA	$p<0.001$ ** *Rho*= 0.636	$p<0.001$ ** *Rho* = 0.761	
Anti-CCP	$p=0.798$ ^{ns} *Rho*= - 0.028	$p=0.533$ ^{ns} *Rho* = - 0.067	$p=0.792$ *Rho* = - 0.029

******: Highly significant correlation using Spearman correlation test

Discussion

RA is an autoimmune disease with unknown etiology, characterized by chronic inflammation of the synovial joints resulting in tissue destruction and joint deformity. RA is thought to be developed due to various genomic, physiological, and biological factors [14,15]. According to the results of this study, most of the patients aged >40 years with a mean age of 49.42±9.46 years. These results are highly consistent with other studies that mentioned RA appearing in middle-aged people, and the most common age to develop RA was ≥40 years [16,17]. Another study reported that RA was more common in those ≤50 years old (Ljung & Rantapää-Dahlqvist, 2016). Usually, RA more commonly appears in middle-aged and advanced people; it is rare in childhood. These might be related to the immune system being highly susceptible to the ageing process, which might be associated with a higher risk of developing autoimmune diseases [19].

Generally, RA is 2 to 3 times more frequent in females than males, and research has shown a substantial correlation between RA and sex hormones. In addition, studies offer convincing evidence that autoimmunity is under genetic control, and genes in sexual chromosomes can support the female prevalence [20]. Hence, in the current study, RA affected females more significantly than men. These findings agree with other studies' results (Ljung & Rantapää-Dahlqvist, 2016; Linauskas *et al.*, 2019).

Regarding the BMI, most of the enrolled patients in this study had higher BMI than controls, without significant differences between both genders. These results did not align with other conducted studies (Ljung & Rantapää-Dahlqvist, 2016; Marchand *et al.*, 2021) but agreed with another study [23].

Moreover, most of the patients in this study had >3 years of RA. These findings agreed with the results of other studies [23,24]. Additionally, moderate disease activity was seen in most patients (63.7%) according to the DAS-28 classification, and these findings are similar to another study [25,26]. Additionally, a significant positive weak correlation was observed between the score of DAS-28 and the duration of RA. In contrast, no significant correlation was seen between the DAS-28 score and the biomarkers of the patients. In most cases, RA is diagnosed after >1 year since this disease is a chronic type and needs time to ultimately develop, produce pain and force the patient to visit a hospital for checking. During RA, the joint's lining becomes inflamed, causing damage to joint tissue that causes long-lasting or chronic pain, unsteadiness, and deformity [27].

Regarding the comparison between the genetic history of RA and the incidence of RA, our study showed that

most patients compared to the control group, had a positive history of RA with a highly significant difference ($p<0.001$). These outcomes align with other studies (Jiang *et al.,* 2015; Deane *et al.*, 2017; Deane & Holers, 2019). Furthermore, the inheritance of RA is around 60%, while the correlation of HLA to heritage is about 11–37%. Moreover, HLA-DRB1-specific genes have also contributed to RA susceptibility [31].

Concerning the smoking status and the incidence of RA, we found a non-significant influence between them, which disagreed with some studies [32,33] but supported the findings of another research [34,35]. Smokers are more vulnerable to RA than non-smokers, which might be due to the pathophysiology of RA in smokers, which disturbs oxidative stress and enhance inflammation, autoantibody formation and epigenetic changes [36].

Commonly, for the detection of RA, the ACR/EULAR 2010 criteria implement RF and anti-CCP biomarkers. Besides, other diagnostic biomarkers that can aid the early detection of RA were developed. It is critical to prevent erosion and stop the disease's progression in the early stages. This is why research has concentrated chiefly on developing serological markers and specific laboratory tests to be used in the early detection of RA [37,38]. Consequently, RF showed higher sensitivity (70.46%) among RA patients. This finding agreed with another study, which found that 59% of patients were sensitive to RF [37], while another study found >50% [39]. Numerous environmental and genetic factors result in loss of tolerance to the proteins with a citrulline residue, which cause the production of autoantibodies like anti-CCP antibody and RF [40]. On the other hand, we found the sensitivity of anti-CCP to be 47.73% which is almost close to the findings of another study [39]; however, it disagreed with another research which found 78.67% [26]. Anti-CCP is considered a more sensitive biomarker for RA diagnosis because of its early expression and high specificity [41].

Most patients showed highly significant differences in ESR and CRP compared to the healthy controls. Other researchers observed similar results [42,43]. Regarding the serum level of all studied biomarkers in the current study (Wnt5a, FGF23, anti-SMA, and Anti-CCP), the highest mean was found in the patient's group than controls, with a significant difference ($p<0.05$) for Wnt5a and FGF23, a highly significant difference ($p<0.001$) for anti-CCP, with a non-significant difference ($p>0.05$) for anti-SMA.

In this respect, another study found that β-catenin dependent Wnt biomarker expression in RA patients [44]. Furthermore, another study mentioned that Wnt5a was a potential candidate biomarker for identifying patients with RA patients with usual interstitial pneumonia. The same research also demonstrated that the circulating Wnt5a might be a risk factor and potential biomarker for assessing interstitial lung disease severity and progression in RA patients [45].

On the other hand, the research mentioned that FGF23 was not associated with bone mineral density but may be associated with local bone loss and disease activity in RA patients [46]. Additionally, serum FGF23 was correlated with inflammation, the disease activity of RA, and abnormal bone absorption and biomarkers in RA patients [8]. Another study showed that FGF23 significantly ($p<0.01$) increased in RA patients, similar to that found by another study [8].

Moreover, anti-CCP expression was also reported to be significantly higher in RA patients, similar to other studies [26,47,48]. Generally, anti-CCP is expressed in RA patients [49] because CCP is absent from joints in normal conditions. Nevertheless, various CCPs are present during different forms of inflammation, including RA. As a result, the joint is directly affected by the antibody produced against them, and their serum levels are used as a marker of RA [48].

Concerning anti-SMA serum levels, the expression was higher in RA patients than in healthy control. These results are in line with the outcomes of Anderson et al., who found anti-SMA to be more highly expressed in RA patients (15.3%) than in normal controls (7.6%) ($0.02>p>0.01$) [11]. These might be due to increased IgG in RA patients. On the other hand, Hae et al. 2010 studied synovial fluid of patients with RA. They found the disease-induced alpha-SMA in human adipose tissue-derived mesenchymal stem cells through a TGF-beta1-dependent mechanism [12].

Moreover, there was no significant correlation in the patient's serum concentration of Wnt5a, FGF23, anti-SMA, and anti-CCP with different age groups, duration of RA and joint involvements. Also, there was no significant difference between symptoms (joint involvement) and RA duration to the patient's age.

Therefore, we speculated that the expression of biomarker levels was unrelated and changed with age in patients with RA. The function of this biomarker effect on generation in RA is still unknown.
The relationship among these remodelling biomarkers was thoroughly analyzed. A solid positive correlation has been detected among all these biomarkers (Wnt5a, FGF-23, and anti-SMA) that might act synergistically in RA's initiations and developments. Still, anti-CCP was not correlated to other biomarkers (Wnt5a, FGF23, and anti-SMA). This finding suggests using the ELISA technique in screening these inflammatory and remodelling biomarkers is a novel finding in RA patients because the relationships among these 4 biomarkers have not been reported previously. Further research is needed to determine the remodelling markers' long-term effects on many cases of RA. Additionally, the serum levels of Wnt5a, FGF-23, and anti-SMA are not reflecting tissue expression.

Conclusions

RA was more common in middle-aged people, especially females. The serum level of Wnt5a, FGF23 and anti-CCP in RA patients was significantly higher than in healthy people. Anti-SMA was expressed in patients but not considerably associated with healthy controls. There was no significant correlation in the patient's serum concentration of Wnt5a, FGF23, anti-SMA and anti-CCP with different age groups, duration of RA and joint involvements. The most commonly observed correlation among biomarkers was between Wnt5a and FGF23, followed by FGF23 and anti-SMA, then Wnt5a and anti-SMA. More studies about the roles of FGF23, Wnt5a and anti-SMA in inflammation are needed to be conducted in the future.

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Conflict of interest

The authors confirm that they are not affiliated with or involved in any organization or entity with financial interests.

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