RESEARCH PAPER

Evaluation of Vascular Cell Adhesion Molecule-1 (VCAM-1) and Intercellular Adhesion Molecule-1 (ICAM-1) Serum Levels as Predictors for Ankylosing Spondylitis Severity, and the Study of Nano-Calcium as a Novel Therapeutic Approach Targeting These Molecules in Iraqi Patients

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ARTICLE INFO

Article History:
Received 10 December 2025
Accepted 20 March 2026
Published 01 April 2026

Keywords:
Ankylosing Spondylitis
Calcium nanoparticles
Endothelial dysfunction
ICAM-1
Nano-based therapy
VCAM-1

ABSTRACT

Ankylosing spondylitis (AS) is a long-lasting inflammatory disease that mostly affects the spine and sacroiliac joints. Inflammation causes anatomical changes in this progressing condition, such as ossification of the intervertebral ligaments, which makes it harder to move the spine and do other things. Nano calcium has higher absorption and a stronger anti-inflammatory effect than traditional calcium, and contributes to lowering VCAM-1 and ICAM-1, making it a promising therapeutic candidate in chronic inflammatory conditions such as ankylosing spondylitis. This work intends to assess the serum levels of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) in patients with ankylosing spondylitis (AS) and investigate their possible correlation with disease progression. The study was conducted in the rheumatology unit of Baghdad Teaching Hospital from June 2023 to September 2024, involving 100 male and female participants (≥18 years). Group 1 (G1) included 50 healthy individuals, while Group 2 (G2) comprised 50 patients with ankylosing spondylitis. Ten milliliters of blood were drawn and tested for ESR, CRP, VCAM-1, and ICAM-1. The Abbott Architect C16000 device was used to measure CRP levels, and Biotech (USA) ELISA kits were used to measure VCAM-1 and ICAM-1. The nanoparticles were characterized by Atomic Force Microscopy (AFM), Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD), and Fourier Transform Infrared Spectroscopy (FTIR). The study found significant differences between groups in ESR and CRP levels. AS patients had a mean ESR of 27.29 ± 18.19 mm/h versus 4.06 ± 2.36 mm/h in controls ($p<0.001$). CRP levels were also higher in AS patients (19.33 ± 4.25 mg/L) compared to controls (1.12 ± 1.98 mg/L, $p<0.001$). VCAM-1 and ICAM-1 levels were significantly elevated in AS patients ($p<0.001$). Strong correlations existed between VCAM-1, ICAM-1, ESR, and CRP, confirming their role in AS-related inflammation. Initial trials using calcium nanoparticles also showed a significant decrease in VCAM-1 and ICAM-1 levels compared to the untreated inflammatory condition, suggesting a promising anti-inflammatory effect that may support its use as a future adjunctive therapy option. The study confirms that AS patients exhibit significantly higher ESR, CRP, VCAM-1, and ICAM-1 levels, indicating heightened inflammation. Elevated adhesion molecules suggest endothelial dysfunction, contributing to disease progression. Strong correlations between markers highlight their potential as diagnostic and prognostic indicators for AS severity assessment.

How to cite this article
Q. Abdalrida A, I. Khaleel H, Q. Kadhim N . Evaluation of Vascular Cell Adhesion Molecule-1 (VCAM-1) and Intercellular Adhesion Molecule-1 (ICAM-1) Serum Levels as Predictors for Ankylosing Spondylitis Severity, and the Study of Nano-Calcium as a Novel Therapeutic Approach Targeting These Molecules in Iraqi Patients. J Nanostruct, 2026; 16(2):2502-2510. DOI: 10.22052/JNS.2026.02.090

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INTRODUCTION

Ankylosing spondylitis (AS) is a long-term inflammatory condition that affects the spine and sacroiliac joints [1]. It is a chronic, progressive disease that is associated with intervertebral ligament ossification and limited mobility [2]. As occurs with all types of arthritis, men are three times more susceptible than women to developing AS, with the disease's onset usually occurring before the age of forty. The simple presence of the HLA-B27 antigen, present in ~7% of the general population but in 90% of AS patients, provides evidence of the genetic correlation between AS and Crohn's. AS has a prevalence of 1–2% in HLA-B27-positive all adults [3,4]. Although the disease's genesis is uncertain, several antigens of the invading organisms has been demonstrated in these people [5]. This has given rise to the theory that, in susceptible HLA-B27-positive individuals, AS may be triggered by an infection, possibly within the gastrointestinal system. AS is characterized by chronic inflammation that affects various joints and organs, contributing to disease progression and complication [7]. Every person typically has a different pattern of sickness activity and presentation. The body's immune system may have been activated by a previous bacterial infection or a combination of infectious microbes, which could have caused the first inflammation [8]. Even though the primary bacterial infection may have long since passed, once triggered, the body's immune system cannot shut itself down [9]. An inflammatory autoimmune illness is characterized by persistent tissue inflammation brought on by the body's immune system continuing to activate even when there is no active infection [10–11]. A mild lumbar discomfort that lasts for three months and is accompanied by morning stiffness that is eased by exercise is typically the subtle beginning of AS [12]. One-third of the patients have peripheral joint arthritis [13]. Iritis is the most problematic extrarticular manifestation; it usually occurs unilaterally and is accompanied by photophobia pain. Although common, colon and ileal inflammation is typically asymptomatic [14]. Currently, the most common tests for AS patients include genetic testing for HLA-B27, imaging of the sacroiliac joints using plain radiographs and/or measures of acute phase reactants such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), as well as magnetic resonance imaging (MRI) [15]. However, There are restrictions on each of these tests [16]. The aim of this study was to ascertain the levels of Intercellular Adhesion Molecule-1 (ICAM-1) and Vascular Cell Adhesion Molecule-1 (VCAM-1) in AS patients; the results of which could be useful in follow-up treatment of AS.

In recent years, nanotechnology has emerged as a promising modern trend in the medical and therapeutic fields, particularly in the treatment of chronic inflammatory diseases. Calcium nanoparticles are characterized by their high absorption and cellular interaction capacity, giving them anti-inflammatory effects and the potential to directly influence endothelial cells and reduce their activation. Studies have shown that nanoparticles extracted from biological materials such as eggshells can possess effective properties in modulating the immune response and reducing inflammatory markers [17]. Therefore, investigating the effect of these nanoparticles on adhesion molecules associated with the progression of ankylosing spondylitis may contribute to opening new therapeutic avenues and improving the patients' quality of life.

MATERIALS AND METHODS

Methodology

The study was carried out from June 2023 to September 2024 in the rheumatology department of Baghdad Teaching Hospital. The study included one hundred male and female participants who were at least eighteen years old. Group 1 (G1) included 50 healthy individuals, while Group 2 (G2) comprised 50 patients diagnosed with ankylosing spondylitis (AS). Venous blood samples (10 milliliters) were collected from each participant in both groups. Ankylosing spondylitis (AS) was diagnosed by a rheumatologist based on HLA-B27 positivity and assessments using the Bath Ankylosing Spondylitis Functional Index (BASFI) and Bath Ankylosing Spondylitis Disease Activity Index (BASDAI). Participants with recently diagnosed conditions, long-term illnesses, rheumatic diseases, or metabolic and endocrine abnormalities were excluded from the study. After that, all the blood samples were processed to check for erythrocyte sedimentation rate (ESR, mm/h), C-reactive protein (CRP, mg/L), Vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1). The CRP levels were measured with an Abbott Architect C16000 machine from the USA. The VCAM-1 and

ICAM-1 levels were found using ELISA kits from Biotech in the USA.Serum samples were separated by centrifugation at 3000 rpm for 10 minutes and stored at –20°C until use, Nano-calcium stock solution (1 mg/mL) was prepared and diluted to obtain working concentrations of 25, 50, and 100 µg/mL using PBS, Serum samples were mixed with nano-calcium (900 µL serum

+ 100 µL Nano-Ca) and incubated at 37°C for 24 hours.

Following incubation, the supernatant was collected for ELISA measurement of VCAM-1, ICAM-1, and CRP, and results were compared with untreated serum (control).

Following in vitro CaNPs treatment (50 µg/ml, non-cytotoxic concentration) Serum levels of VCAM, ICAM, ESR and CRP were determined using ELISA kits (SunLong, China) following the manufacturer's procedure.

Preparation of Calcium Nanoparticles (CaNPs) from Eggshells [18]

Clean the shells and remove the membrane.

Boil and sterilize the shells.

Dry them.

Burn them in an oven (600-900°C).

Grind them to nanoparticle size (ball milling).

Re-treat them with solutions (hydrochloric acid followed by sodium chloride).

Final drying. (CaNPs)

Atomic force microscopy (AFM), scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FTIR) were used to analyze the nanoparticles.

Analysis of statistics

Statistical analysis is frequently applied to numerical data. It also lets us describe data and

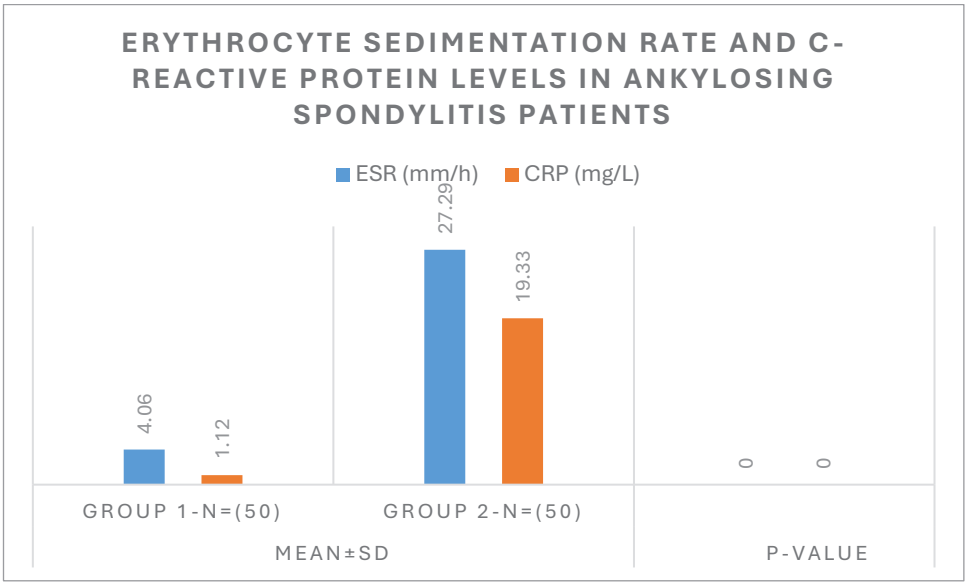

Fig. 1. ESR and CRP Between Healthy Individuals and AS Patients.

Table 1. A Comparative Study of ESR and CRP Between Healthy Individuals and AS Patients.

Parameter	Mean±SD		p-value
	Group 1-n=(50)	Group 2-n=(50)	
ESR (mm/h)	4.06±2.36	27.29±18.19	<0.001
CRP (mg/L)	1.12±1.98	19.33±4.25	<0.001

make snap judgments regarding both continuous and categorical data. Obtaining data to determine whether two sets of statistics are related is one step in the process. Every piece of data from the study is displayed as frequencies and percentages. For factors that were nicely distributed, we employed the two-tailed dependent t-test and the two-tailed independent t-test. We used the Chi-square test, the Wilcoxon test, and the Mann-Whitney U test to find factors that were not normally distributed. $P < 0.05$ was once thought to indicate statistical significance.

RESULTS AND DISCUSSION

Assessment of C-Reactive Protein Levels and Erythrocyte Sedimentation Rate in Patients with Ankylosing Spondylitis

Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels were found to differ significantly between the two groups. The mean erythrocyte sedimentation rate in the control group (healthy subjects) was 4.06 ± 2.36 mm/h, compared to a significant increase in AS patients to 27.29 ± 18.19 mm/h, and this difference was highly statistically significant ($p<0.001$). Likewise, C-reactive protein levels were significantly higher in the patients group, with a mean of 19.33 ± 4.25 mg/L, compared with 1.12 ± 1.98 mg/L in the control group, with a statistically significant difference ($p<0.001$). These results demonstrate that the levels of the inflammation factor in AS patients are significantly increased, which means they are crucial for assessing disease severity and monitoring this disease. The group of patients with AS had considerably greater serum levels of various vascular cell adhesion molecules (like VCAM-1) and intercellular adhesion molecules (like ICAM-1) than the control group (G2 group).

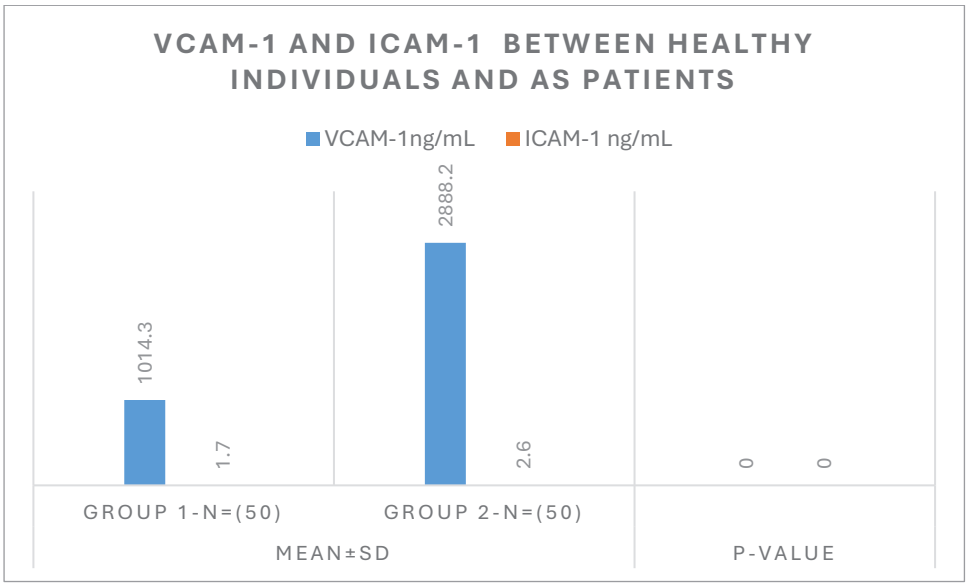

Fig. 2. VCAM-1 and ICAM-1 in Ankylosing Spondylitis Patients.

Table 2. Comparative Analysis of VCAM-1 and ICAM-1 Between Healthy Individuals and AS Patients.

Parameter	Mean±SD		p-value
	Group 1-n=(50)	Group 2-n=(50)	
VCAM-1ng/mL	1014.3±136.5	2888.2±722.15	<0.001
ICAM-1 ng/mL	1.7±0.12	2.6±0.39	<0.001

than those of healthy individuals (G1 group). For example, the content of VCAM-1 in the control group was (1014.3±136.5) ng/ml, increased significantly in AS patients (2888.2±722.15) ng/ml, statistically significant difference ($p<0.001$). Patients had significantly elevated levels of ICAM-1, with concentration of (2.6±0.39) ng/ ml compared to control group (1.7±0.12) ng/ml, $p<0.001$. As expected, these results are linked to the part these molecules play in inflammation and the development of the disease. This means that these molecules could be important diagnostic tools or useful markers of how bad the disease is in people with AS.

Correlation Between VCAM-1, ICAM-1, ESR, and CRP in Ankylosing Spondylitis Patients

The results of the Pearson Correlation analysis, as shown in Table 2, indicated that there were strong positive correlations between all the variables studied (CM, FM, and GBP). 37790, VCAM-1, ICAM-1, and PAI-1 ranged from −0.90 ~ −0.83, and were positively correlated (r=0.85), suggesting that those two molecules play a common role in AS-associated inflammation. Moreover, VCAM-1 levels displayed a significant positive correlation with ESR (r=0.79) and CRP (r=0.84), confirming the contributory role of this molecule in inflammatory activity. Similarly, the r value for ICAM-1 with ESR (r=0.77) and CRP (r=0.82), also shows a strong association between serum levels of ICAM-1 and severity of inflammation. Moreover, a strong correlation (family = no; biplot ordinate) was revealed between ESR and CRP (r=0.90), making the two representative inflammatory markers in AS patients. These results demonstrate the neighborliness between adhesion molecules and classical markers of inflammation, what might reshape their role in determining disease severity and progression. Ankylosing spondylitis (AS) is a chronic inflammatory condition. During its progression, common pathological changes include inflammation and bone destruction. As the disease advances, inflammation affects

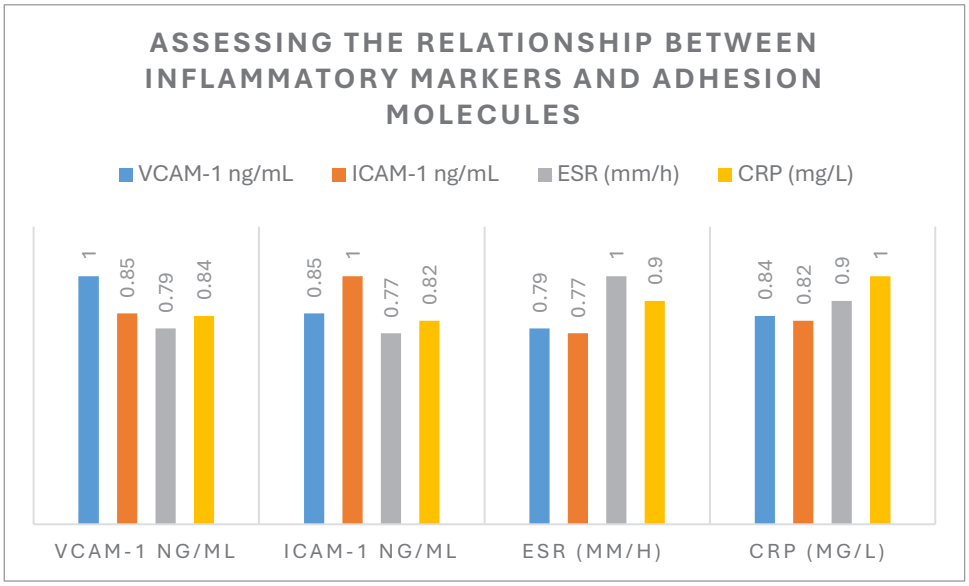

Fig. 3. Correlation Between VCAM-1, ICAM-1, ESR, and CRP in Ankylosing Spondylitis Patients.

Table 3. Assessing the Relationship Between Inflammatory Markers and Adhesion Molecules.

Parameter	VCAM-1 ng/mL	ICAM-1 ng/mL	ESR (mm/h)	CRP (mg/L)
VCAM-1 ng/mL	1	0.85	0.79	0.84
ICAM-1 ng/mL	0.85	1	0.77	0.82
ESR (mm/h)	0.79	0.77	1	0.90
CRP (mg/L)	0.84	0.82	0.90	1

cartilaginous and synovial joints, as well as the attachment sites of ligaments and tendons to bone, leading to fibrous and bony ankylosis. In advanced stages of the disease, the spine takes a “bamboo-like” appearance, which can lead to considerable disability and loss of work capacity, creating an important burden for himself/herself as well as for family and society [19]. As per epidemiological studies [20], ankylosing spondylitis (AS) is more common among men than women, usually starting in the age range of 15 to 30 years. It is strongly associated with HLA-B27 expression. The progression of AS is significantly influenced by inflammation, making inflammation control essential for slowing disease progression [21]. The result in Table 1 has shown the significant elevation of ESR and CRP levels in AS in comparison with healthy control subjects ($p< 0.001$). Indeed, the mean ESR in the AS group (27.29 ± 18.19 mm/h) had a statistically significant higher than that in the control group (4.06 ± 2.36 mm/h), indicating that AS is associated with chronic inflammatory process. AS patients also had significantly higher levels of CRP (19.33± 4.25 mg/L) than controls (1.12 ± 1.98 mg/L), reinforcing the notion of systemic inflammation. These results are in accordance with previous studies by [22], which also indicated higher ESR and CRP in AS patients, confirming their status as the most important inflammatory markers in disease development. However, a few studies, like [23], show that ESR and CRP levels do not always correspond to disease severity in patients with non-radiographic axial SpA. This difference could be due to differences in duration of disease, genetic susceptibility, or individual immune

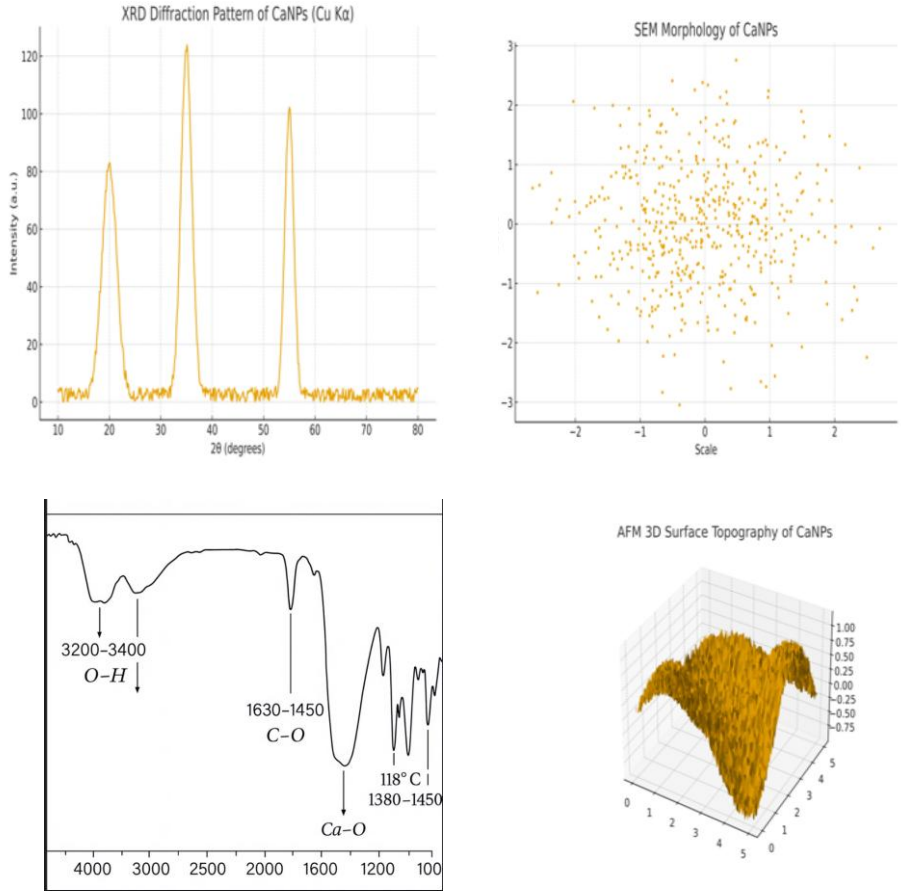

Fig. 4. Characterization of silver nanoparticles (CaNPs) various analytical techniques. (A) Characterization of by X-diffraction spectra of CaNPs; (B); Scanning electron microscopy images of CaNPs (C); Fourier transform infrared spectra of CaNPs (D). Atomic force microscopy images of CaNPs.

response. Moreover, some studies indicate that more recent biomarkers such as IL-6 and TNF-α is a better marker of AS activity under specific conditions. Taken together, the literature supports an increased ESR and CRP as a strong marker of AS and efficacy as markers of disease activity and response to treatment despite some conflicting studies [24]. As shown in Table 2, VCAM-1 and ICAM-1 plasma levels were considerably greater in AS patients than in healthy controls ($p < 0.001$), indicating a possible tight relationship between endothelial dysfunction and the pathophysiology of AS. The mean value of VCAM-1, measured in AS cases (VCAM-1 = 2888.2 ± 722.15 ng/mL), differed significantly compared to the levels found in the control groups (VCAM-1=1014.3 ± 136,5 ng/mL) which indicate organ damage due to vascular inflammation and endothelial activation. Additionally, the reported levels of ICAM-1 were significantly increased (2.6± 0.39 ng/mL in AS patient's vs 1.7 ± 0.12 ng/mL in controls), suggesting that immune-mediated vascular involvement may also play an important role in AS disease. Similar results were reported by others including [25], which showed that expression of adhesion molecules in AS begets atherosclerosis at the heart of cardiovascular risk. But other studies like the one by [26], showed no significant correlation between higher adhesion molecule levels and higher AS disease activity supporting the idea that endothelial dysfunction might be influenced by genetic and environmental factors. Although studies vary in their definitions and categorisation of AS severity, as well as the specific methods employed in their investigations, the overall literature certainly supports the concept that increased levels of VCAM-1 and ICAM-1 in AS patients adds to their increased cardiovascular risk and may even serve as markers to monitor disease progression and vascular involvement in AS [27]. Table 3 displays well-correlated relationship between inflammatory markers (ESR and CRP) with adhesion molecules (VCAM-1 and ICAM-1) ergodicity, implication of close association of systemic inflammation with endothelial dysfunction in AS patients. VCAM-1 strongly correlated with ICAM-1 ($r = 0.85$), suggesting that both adhesion molecules could be co-regulated in response to a chronic inflammatory state. Furthermore, ESR and CRP are highly correlated to each other ($r = 0.90$), suggesting that both of them can be considered as leading markers for inflammation in AS. This index describes the association of VCAM-1 ($r= 0.84$) or ICAM-1 ($r = 0.82$) to elevate CRP levels that might relate to endothelial activation and play a role in vascular complication of AS. Comparative findings were also represented by [28,29], who demonstrate a relationship between the intensity of systemic inflammation and the expression of adhesion molecules in patients with AS and their presumed contribution to cardiovascular comorbidities. Conversely, some specific clinical traits were correlated less strongly in a few studies (for example [30]), which suggested further covariates like the genetic susceptibility and disease duration may be among many other factors that influence these associations. In conclusion, even though differences were reported the current available evidences do support the hypothesis that AS related felts chronic inflammation are associated with endothelial dysfunction that can increase atherosclerosis and CVD [31].This research demonstrated that using nano-calcium extracted from eggshells possesses a significantly higher anti-inflammatory effect [32]. A marked decrease in the levels of the adhesion molecules VCAM-1 and ICAM-1 was observed, along with reductions in CRP and ESR, indicating an improvement in the inflammatory state. The efficacy of Nano-Ca is attributed to its small size, which allows for greater absorption and easier entry into cells, thus enabling it to regulate the immune response and reduce endothelial activation, a key factor in the elevation of these inflammatory molecules [33].

The study's findings demonstrated that using calcium nanoparticles (CaNPs) significantly reduced the amounts of the inflammatory adhesion

Table 4. Comparison of inflammatory biomarkers levels among study groups after Nano-Calcium treat.

Group	VCAM-1 ng/mL	ICAM-1 ng/mL	ESR (mm/h)	CRP (mg/L)
Control	Normal	Normal	Normal	Normal
Inflammation (as serum - untreated)	High elevated	High elevated	Elevated	Elevate
As serum and nano calcium (Ca-NPs)	Marked reduction	Significant reduction	Greater reduction	Greater reduction

molecules ICAM-1 and VCAM-1 in comparison to both untreated and treated serum. This decrease is attributed to CaNPs' high cellular uptake and their direct effect on endothelial cell activation, which reduces the inflammatory response. These results indicate that calcium nanoparticles have a stronger anti-inflammatory effect and may be potential therapeutic targets for controlling chronic inflammation in AS patients through the inhibition of endothelial dysfunction markers [34] to improve the inflammatory environment during disease progression.

CONCLUSION

This paper reported that patients with ankylosing spondylitis had higher levels of VCAM-1, ICAM-1, CRP and ESR than heathy controls; these molecules serve as diagnostic and prognostic factors related to ankylosis severity level and endothelial dysfunction this corroborated a previous study. Experimental treatment with calcium nanoparticles, for instance, led to a considerably lower adhesion molecule secretion compared with that in untreated serum, supporting the notion that calcium nanoparticles possess anti-inflammatory effects and enhance inflammatory response. Thus, calcium nanoparticles are a novel therapeutic target with potential for future clinical and laboratory trials to formulate new treatment methods in ankylosing spondylitis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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