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**Original Reserach Article**


# Investigate the Genetic Variations of the Vitamin D Receptor SNP (C>A rs7975232) in the Individuals Diagnosed with Rheumatoid Arthritis


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**Abstract:** Rheumatoid arthritis (RA) is a persistent autoimmune condition that impacts the joints, featuring a gradual and symmetrical inflammation in the affected joints. This inflammation leads to the destruction of cartilage, erosion of bone, and eventual disability. The objective of this study is to assess the VDR SNP (rs7975232) and its potential correlation with fatty acid synthase (FAS) and IL-17A in Iraqi patients diagnosed with RA. In this study, demographic characteristics were analyzed for 90 subjects, comprising 45 patients with rheumatoid arthritis (RA) and 45 control subjects. The results revealed statistically significant increases in total cholesterol and LDL in the study group (p-values $< 0.0001$ and $< 0.001$, respectively). Conversely, HDL exhibited a significant decrease (p-value $< 0.001$), while TG and VLDL showed non-significant differences (p-values 0.1 and 0.09, respectively). The study further concludes a notable disparity in the levels of the fatty acid synthase (FAS) enzyme, with the patient group ($0.93 \pm 0.53$) demonstrating a higher level compared to the control group ($0.74 \pm 0.45$) (p-value: 0.001). Additionally, the study identified a significant difference in the levels of IL-17A (pg/ml) between patients ($166 \pm 13$) and the control group ($87 \pm 5$) (p-value: 0.001), with the patient group exhibiting higher IL-17A levels. In conclusion, assessing both FAS and IL-17A may serve as a valuable method for the characterization and monitoring of subjects with RA.



**Keywords:** Rheumatoid arthritis; vitamin D receptor, Fatty acid synthase; IL-17A; lipid profile.




## INTRODUCTION

Rheumatoid arthritis (RA) is a persistent autoimmune disorder that primarily affects the joints. The condition is characterized by a progressive, symmetrical inflammation of the joints, leading to the destruction of cartilage, bone erosion, and resultant disability [1]. Initially impacting only a few joints, RA can later involve numerous joints, accompanied by common extra-articular symptoms [2]. Clinical manifestations of RA vary significantly between the early stages and inadequately treated later stages of the disease. Early-stage RA is marked by general symptoms such as fatigue, flu-like sensations, swollen and tender joints, and morning stiffness. This stage is associated with elevated levels of C-reactive protein (CRP) and an increased erythrocyte sedimentation rate (ESR) [3]. While the exact cause of RA remains unknown, both genetic and environmental factors have been implicated in its development [4]. Risk factors include smoking, obesity, exposure to UV light, sex hormones, certain medications, alterations in the gut, mouth, and lung microbiome, periodontal disease, and infections [5-8]. The pro-inflammatory cytokine IL-17A, produced by Th17 cells, plays a pivotal role in RA progression. IL-17A stimulates the production of inflammatory cytokines (IL-6, IL-8, and GM-CSF), induces neutrophil recruitment, and contributes to local inflammation, bone erosion, cartilage destruction, and neoangiogenesis in RA patients [9, 10]. Additionally, IL-17A promotes the production of matrix metalloproteinase (MMP)-1 by synoviocytes, leading to cartilage degradation [11]. n the context of RA pathogenesis, IL-17A enhances endothelial cell migration and induces the production of vascular endothelial growth factor (VEGF) by synovial fibroblasts [12, 13]. Fatty acid synthase (FAS), a pivotal enzyme encoded by the FASN gene in humans [14], is a multi-enzyme protein system facilitating the synthesis of

palmitate (C16:0), a long-chain saturated fatty acid, from acetyl-CoA and malonyl-CoA in the presence of NADPH [15]. Fatty Acid Synthase (FAS) is a critical enzyme involved in the biosynthesis of fatty acids, playing a fundamental role in cellular lipid metabolism. Encoded by the FASN gene in humans, FAS is a multifunctional protein responsible for catalyzing the stepwise synthesis of long-chain fatty acids, primarily palmitate (C16:0). The enzymatic process involves the conversion of acetyl-CoA and malonyl-CoA, facilitated by NADPH as a reducing agent. FAS is unique in that it is not a singular enzyme but a complex enzymatic system comprised of two identical 272 kDa multifunctional polypeptides. This structural complexity allows for the sequential passage of substrates between various functional domains, enabling the intricate and controlled synthesis of fatty acids. The synthesized fatty acids serve as essential components of cell membranes, energy storage molecules, and precursors for various bioactive lipid molecules. As such, FAS plays a crucial role in maintaining cellular homeostasis, influencing cell proliferation, differentiation, and overall lipid metabolism. This study aims to assess FAS, IL-17A, and the lipid profile in Iraqi RA patients, seeking insights into potential interconnections among these factors and the disease's progression.

## MATERIAL AND METHODS

**Total DNA Extraction**

The genomic DNA was isolated from the peripheral blood of subjects using Favorgene® kit Genomic DNA Purification Kit depending on protocol provided by manufacture. Only DNA samples with adequate purity ratios (A260/A280=1.7 – 2) were used for subsequent analyses. Until analysis, DNA was stored at -20°C. VDR SNP (*ApaI* (rs7975232)) detection was done by polymerase chain reaction through restriction fragment length polymorphism (PCR-RFLP) technique by using specific primer sequences (F: CAGAGCATGGACAGGGAGCAA and R:G AACTCCTCATGGCTGAGGTCTC).

**Measurement of Serum Lipid Profile:**

"""Spectrophotometric methods were employed, following the manufacturer's instructions, to assess levels of total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL)."

**Serum of fatty acid synthase FAS and IL-17A measurementmethod was used to determ**

Avidin-Horseradish Peroxidase (HRP) and a biotinylated detection antibody specific to the Human FAS and IL-17A combo were then added and incubated in each microplate. After that, the free parts were cleaned off. Each well received the substrate solution; only the wells containing the Human FAS and IL-17A, biotinylated detector antibody, and the Avidin-HRP conjugation all had a blue tinge . The enzyme-substrate process was stopped by adding the stop solution, which is why the color changed to yellow. Using spectrophotometry, the optical density (OD) was determined with a wavelength of 450 nm ± 2 nm. The concentration of human FAS was discovered to be proportionate to the OD value.

**Statistical Analysis**

"The statistical analysis for this prospective study was carried out utilizing the Statistical Package for the Social Sciences (SPSS) version 20.0 and Microsoft Excel 2013. A comparison between two groups was performed using the independent sample t-test, while ANOVA was employed for comparisons among more than two groups. Categorical data were presented as counts and percentages, with the association between variables estimated using the Chi-square test. The predetermined threshold for accepting statistical significance was set at $< 0.05$.

## RESULTS

"The outcomes of the PCR-RFLP assay, involving amplification and digestion with the restriction enzyme ApaI, for the VDR gene, revealed two distinct alleles. The aa (A/A) allele showed a single band of 746 bp, whereas the AA (C/C) allele showed 2 bands with molecular diameters of 532 and 214 bp. ApaI (RE) digested the Aa (A/C) an allele, producing three different bands at 746, 532, and 214 bp.

Table 1 summarize the value of the VDR SNP (C>A rs7975232) frequencies in the RA group and their relationships with age and BMI.

**Table 1: The correlation between VDR SNP C>A rs7975232 in RA group with Age and BMI**

| Parameters | | Genotypes/ RA n =45 (%) | | | P-value |
|---|---|---|---|---|---|
| | | AA 19(42%) | Aa 11(24%) | aa 15(34%) | |
| Age | <50 20(55%)<br>≥50 26(45%) | 10(22)<br>9(20) | 7(16)<br>4(9) | 5(11)<br>10(20) | 0.032* |
| BMI | <25 29(64%)<br>≥25 16 (36%) | 11 (24)<br>8 (18) | 6 (13)<br>5 (11) | 8 (18)<br>7 (16) | 0.082 |

All subjects are categorized depending on the process of fragmentation of amplicons of VDR SNP (C>A rs7975232) gene being AA genotype for A allele as homozygous, Aa genotype as heterozygous, and aa genotype as homozygous, as shown in table 2.

**Table 2: Genotyping of VDR SNP (C>A rs7975232) and allele frequency**

| Genotypes | CONT n=45 (%) | RA n= 45(%) | Total | OR (CI 95%) | p-value |
|---|---|---|---|---|---|
| AA | 9 (20) | 19 (42) | 28 | 1.0 (Reference) | |
| Aa | 10 (22) | 14 (31) | 24 | | |
| aa | 26 (58) | 12 (27) | 38 | 2.88 (1. 34-4.54) | 0.000* |
| **Total** | 45 | 45 | 90 | | |
| **HWE (Alleles frequency) (p+q=1)** | | | | | |
| A | 39% | 59% | | 2.12 (1.21-5.34) | 0. 000* |
| a | 61% | 41% | | | |

Regarding the VDR (ApaI SNP rs7975232), among CONT subjects, the genotypic frequencies were 20% (n = 9) for the normal genotype (AA), 22% (n = 10) for the heterozygous genotype (Aa), and 58% (n = 26) for the homozygous genotype (aa). The A and a alleles exhibited frequencies of 39% and 61%, respectively. In the RA group, corresponding frequencies included 42% (n = 19) for the normal genotype (AA), 31% (n = 14) for the heterozygous genotype (Aa), and 27% (n = 12) for the homozygous genotype (aa). Allele frequencies for A and a were 59% and 41%, respectively.

The findings of the current study reveal statistically significant differences in genotyping frequencies (AA, Aa, and aa) of VDR SNP (C>A rs7975232) (p-value < 0.05) between the RA and CONT groups. Based on the results, the presence of A or a alleles in VDR ApaI appears to be a risk factor for OST development when compared to women with Aa genotypes. The genotyping frequencies, by Hardy-Weinberg equilibrium (HWE), for ApaI SNP in VDR among OST and control groups are depicted in the figure 1.

The study looked at the demographics of the 90 participants, 45 of whom had RA and the remaining 45 of whom were control subjects.

According to Table (3), women are more likely than men to have RA (68% vs. 32%).

**Table 3: Distribution of genders in the CONT and RA groups**

| Sex | Study groups | | P-value |
|---|---|---|---|
| | RA patients | Control | |
| Female | 31 | 23 | 0.677 $^{NS}$ |
| % | 68% | 51% | |
| Male | 14 | 22 | |
| % | 32% | 49% | |
| **Total** | **45** | **45** | |

Tables 4 and 5 compare the mean± SD of FAS and IL-17A according to AA, Aa, and aa genotypes in order to examine the impact of VDR SNP (C>A rs7975232) in various RA and CONT group genotypes.

**Table 4: The comparison of FAS levels (ng/ml) across all study group genotypes**

| Genotype (Co-dominant) | (FAS ng/ml) levels in CONT (Mean ± SD) | (FAS ng/ml) levels in RA (Mean ± SD) | p-value |
|---|---|---|---|
| AA | 0.9±0.009 | 1.4±0.9 | 0.000 |
| Aa | 1.26±0.004 | 1.69±0.8 | 0.000 |
| aa | 1.05±0.005 | 1.91±0.4 | 0.000 |
| AA vs. Aa+ aa (Dominant) | 1.04±0.001 | 1.87±0. 5 | 0.000 |
| AA+ Aa vs. aa (Recessive) | 0.78±0.008 | 1.55±0.7 | 0.000 |
| AA+ aa vs. Aa (over dominant) | 0.69±0.002 | 1.77±0.7 | 0.000 |

**Table 5: Comparing the IL-17A levels (pg/ml) in each study group's genotype**

| Genotype (Co-dominant) | (IL-17A pg/ml) levels in CONT (Mean ± SD) | (IL-17A pg/ml) levels in RA (Mean ± SD) | p-value |
|---|---|---|---|
| AA | 105.3±11.2 | 188.8±12.7 | 0.000 |
| Aa | 101.6±12.2 | 199.8±12.9 | 0.000 |
| aa | 100.5±12.1 | 206.7±12.1 | 0.000 |

| Genotype (Co-dominant) | (IL-17A pg/ml) levels in CONT (Mean ± SD) | (IL-17A pg/ml) levels in RA (Mean ± SD) | p-value |
|---|---|---|---|
| AA vs. Aa+ aa (Dominant) | 99. 4±9.1 | 196.87±12.2 | 0.000 |
| AA+ Aa vs. aa (Recessive) | 107. 8±12.2 | 198.4±12.3 | 0.000 |
| AA+ aa vs. Aa (over dominant) | 105.9±12.1 | 206.9±10.5 | 0.000 |

In Figure 2, depicting the distribution of patients based on the presence or absence of positive Rheumatoid Factor (RF), the majority of patients (68%) exhibit a positive RF, whereas the remaining subset (32%) has a significant P-value < 0.05 and a negative RF. There are two subgroups within the control group: the first grouping, which makes up around 10% of the control group, has a positive RF, whereas the other 90% of the control group has a negative RF.

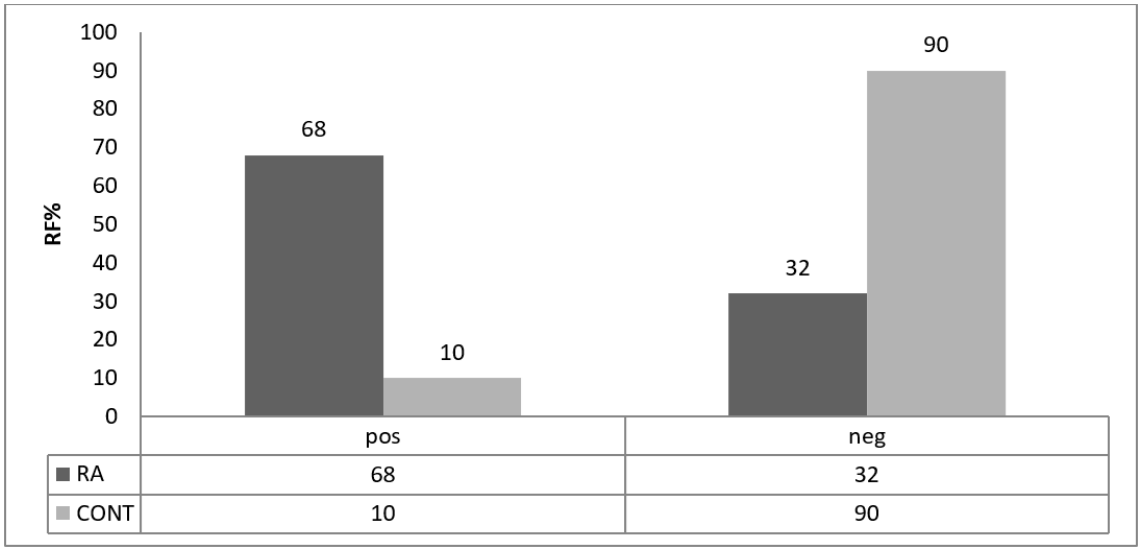


**Figure 2: RF percent in the CONT and RA groups**

The results indicate a statistically significant rise in LDL as well as cholesterol levels in the present study group (p-value < 0.001 for both), while HDL is notably found to have a significant decrease (p-value < 0.001). In contrast, there were no statistically significant differences for TG and VLDL, with p-values of 0.1 and 0.09, respectively. These findings are presented in figure 3.

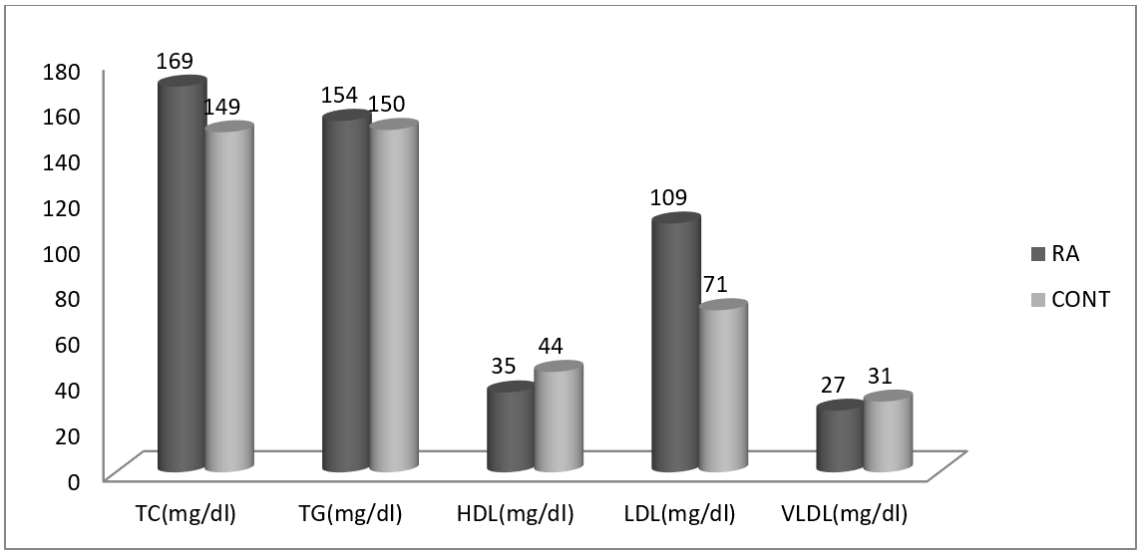


**Figure 3: Lipid profile results**

Based on the analysis of the current study, the levels of FAS and IL-17A in the patients (1.53 ± 0.48) with those in the control group (0.69 ± 0.13) differ significantly, with a P-value of 0.001, as shown in Figure 3. Interestingly, the sick group has higher levels of IL-17A and FAS than the control group.

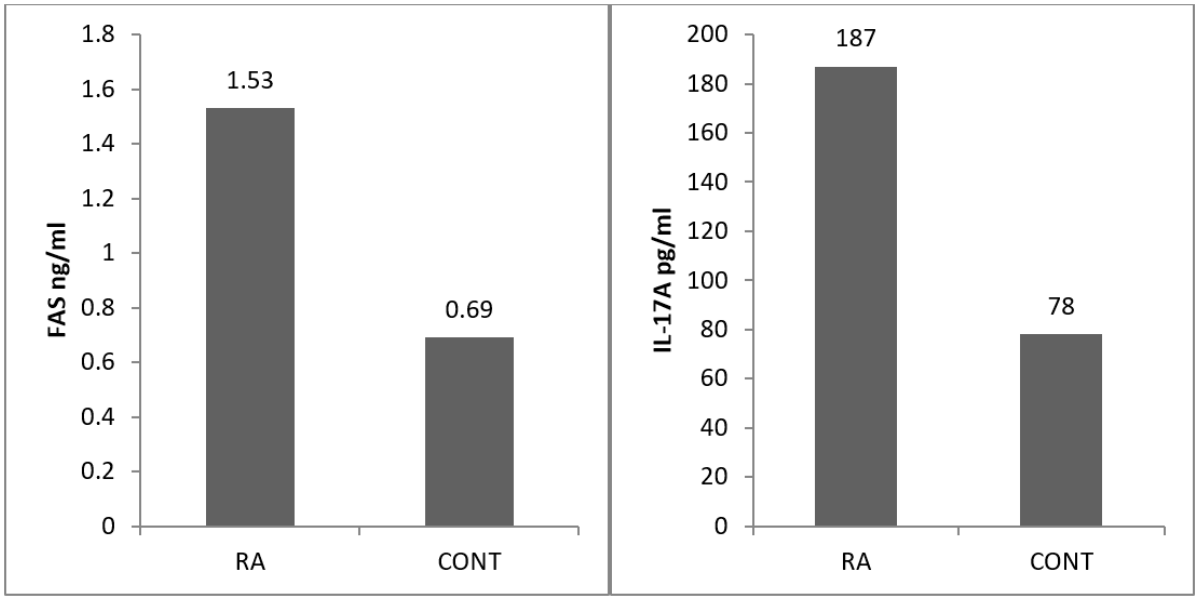


**Figure 3**: **FAS (ng/ml) and IL-17A (pg/ml) levels in the RA group and CONT group**

## DISCUSSION

Rheumatoid arthritis (RA) is a progressive inflammatory condition that can cause joint degeneration if left untreated. Certain clinical and laboratory data can be used to predict the prognosis in RA, and the revised RA categorization criteria offer the chance for earlier treatment [17]. From the beginning of arthritis to certain rheumatic disorders like RA, Inflammatory arthritis sufferers may experience multiple phases [18]. The purpose of this study is to look into how IL-17A and FAS function as predictive markers in the diagnosis of RA. The study examines how the VDR SNP (C>A rs7975232) affects the various RA and CONT group genotypes. The AA, Aa, and aa genotypes are used to compare the mean± SD of FAS and IL-17A. With a p-value of 0.001, FAS and IL-17A levels in the patients (1.53 ± 0.48) as well as those in the control group (0.69 ± 0.13) differ significantly, according to the results. FAS and IL-17A levels are higher in the sick group than in the control group. According to Tański *et al.*, [19], some necessary fatty acids for the formation of eicosanoid compounds that have anti-inflammatory qualities. The study suggests that the standard of care for RA patients should include a diet rich in long-chain unsaturated acids in addition to medicines. An anti-inflammatory diet that includes fish oil has been shown in numerous trials to help RA sufferers with their joint pain. Cod liver oil dramatically decreased the need for NSAIDs in RA patients, according to a research by Galarraga *et al.*, [25]. The present investigation revealed non-significant changes in TG and VLDL (p-values of 0.1 and 0.09, respectively), a statistically significant increase in cholesterol and LDL (p-value < 0.001), and a substantial drop in HDL (p-value < 0.001). Other investigations have shown that fatty acid changes in RA patients after a seven-day fast limit in vitro T-lymphocyte proliferation. In vitro, lymphocyte proliferation is greatly impacted by the FFA mixture concentration and the ratio of unsaturated to saturated fatty acids (p-value < 0.0001) (26,27). IL-17 as a possible means of determining that treatments which target IL-17 will help RA sufferers [28]. The creation of predictive indicators of reaction has become crucial in this setting. To sum up, evaluating FAS and IL-17 may aid in the diagnosis and monitoring of RA.

## CONCLUSION

Together with lipid profiles and IL-17A, the FAS assay was added as the primary marker, suggesting a novel method for diagnosing and monitoring RA patients.

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