Is There a Feasible Link between Vitamin D Receptor Genotypic and Allelic Frequencies with Analytical Biomarkers of Rheumatoid Arthritis Disease?

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Summary  Rheumatoid arthritis (RA) is one of the most widespread autoimmune disorders and it has a genetic background with a variety of genes affecting the degradation of the immune system. Along these lines, we assessed the relationship between the BsmI, and FokI VDR polymorphisms and inflammable records identified with infections activity. Such as interleukins (IL-6, IL-8), hypoxia inducible factor-alpha (HIF-α), soluble receptor of advanced glycation end product (sRAGE), oxidized low-density lipoprotein cholesterol (oxLDL), neutrophil gelatinase-associated lipocalin (NGAL) and procollagen N-propeptide of type III collagen (P3NP) and the allelic frequencies of BsmI VDR rs1544410 and FokI VDR rs2228570 polymorphism on the RA. Total of 131 subjects [70 RA patients and 61 age and sex matched apparently healthy controls (HC)] were monitored for inflammatory biomarkers using ELISA. All patients were screened for the BsmI and FokI using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The all biomarkers were significantly higher in RA patients in comparison with HC. There were positive correlations between NGAL, oxLDL and s-RAGE, oxLDL. On BsmI, ‘GG’ and ‘AG’ genotypes were significantly associated with high RA activity as well as the frequency of genotypes ‘AG & GG’ were higher in high activity RA as compared to low RA activity. However on FokI, was observed that in high activity patients the frequency of ‘CC’ & ‘CT’ was more prevalent as compared to low activity ones. These outcomes support the immunoregulatory role of vitamin D which is associated with several inflammatory diseases, signifying a credible anti-inflammatory role in perturbation of the RA.

Key Words  VDR, polymorphism, rheumatoid arthritis, BsmI, FokI

Rheumatoid arthritis (RA) is one of the most prevalent autoimmune diseases worldwide, affecting 0.5–1% of the adult population, with a sex bias towards women from 30 to 50 y of age (1–3). A variety of genes is involved in the immune balance breakdown associated with RA (4). Vitamin D3 is known to be involved in calcium homeostasis. It can control bone turnover and increase bone reabsorption and improvement (5). Activated vitamin D performs its cellular functions after binding to the nuclear vitamin D receptor (VDR). Several polymorphisms are conveyed for the VDR gene (6). Medical studies focused on two VDR single-nucleotide polymorphisms (SNPs), namely, BsmI-rs1544410 G>A placed in intron 8 and FokI-rs2228570 C>T located in exon 2 were explored association of VDR polymorphisms with diseases (7). However, the molecular link between vari-
ous polymorphisms of VDR and disorders remains uncertain.

Lipoprotein-related disorders play a key role in the development of atherosclerosis and autoimmune diseases. There is evidence proving the importance of lipid profile assessment in joint idiopathic arthritis (JIA). Oxidized low-density lipoprotein cholesterol (oxLDL) promotes inflammation, cell damage, the production of inflammatory cytokines, cell adhesion, and apoptosis (8).

Novel serum indicators involved in bone turnover and remodelling have been recently discovered, including the C-terminal propeptide of type I procollagen (PICP), N-terminal propeptide of type I procollagen (PINP), and C-terminal pyridinoline cross-linked telopeptide of type I collagen (ICTP). Serum PICP and PINP levels are reflected to be the most abundant protein in bone (9). P3NP is chiefly synthesised during growth and development and inflammation. Type III collagen is synthesised as a procollagen molecule with non-collagenous aminopropeptide P3NP (P3NP) and carboxypropeptide (P3CP) that are concealed into the extracellular space and can be measured in both serum and synovial fluid (9, 10).

Vitamin D is reflected to have an anti-inflammatory outcome and has been shown to apply a defensive effect beside the inflammatory actions of advanced glycation end products (AGEs). The circulating receptor of AGE (RAGE) acts as a decoy receptor and affords protection from autoimmune diseases (11).

There are a few cytokines that have crucial roles in the constant aggravation of joints and related ligaments and causes bone distortion. IL-6 is responsible for formation and separation of different resistant cells. These cytokines have been the focus of various studies as targets of remedial treatment of RA. In the serum of RA patients, elevated levels of IL-6 and their receptors are associated with the disease severity and radiological joint damage (11, 12). Numerous genetic studies on key proinflammatory cytokine genes have reported the role of communal genetic discrepancy in relation to RA risk and disease severity index (13).

Even though the aetiology of RA is not known, ongoing studies show that lipocalin 2 (LCN-2), also called the neutrophil gelatinase-associated lipocalin (NGAL), is a biomarker that has a positive relationship with weight index and different metabolic disorders (14).

Hypoxia-inducible factors (HIFs) are heterodimeric transcription factors, which play a critical role in inciting cell reactions to hypoxia. In macrophages, HIF-1α increases cell motility and the expression of pro-inflammatory cytokines (15).

Although the precise cause of RA remains unknown, recent results suggest a genetic basis for disease progression. Therefore, our hypothesis is aimed to test the alteration in biomarker levels according to disease severity (Fig. 1) and to estimate the genotypic and allelic frequencies of the RA severity groups of two genotypes (BsmI VDR rs1544410 and FokI VDR rs2228570).

**SUBJECTS AND METHODS**

**Subjects.** Seventy patients fulfilling the 2010 ACR-EULAR classification criteria for RA (16), were recruited from the clinic of Rheumatology, Rehabilitation and Physical Medicine Department, Assiut University Hospital. Complete history was recorded, and thorough general and musculoskeletal examination was performed. Various basal characteristics and parameters such as age, sex, disease duration, age of onset, duration of morning stiffness, presence of extra-articular manifestations and their current treatment were recorded. The disease activity in RA patients was assessed by the 28 joint count Disease Activity Score (DAS 28) using the number of swollen and tender joints, erythrocyte sedimentation rate (ESR) and patient’s global status, and pain evaluated by the visual analogue scale (VAS) range from 0 to 100 mm. Sixty-one age- and sex-matched healthy individuals from the hospital personnel, undergraduates, medical and nursing staff were included as the control group. Exclusion criteria for all subjects were conditions and comorbidities that affect lipid profile: cholestatic liver disease, obesity (body mass index > 30 kg/m²), familial hypercholesterolemia, hypertriacylglyceridemia, diabetes mellitus, thyroid disease, Cushing’s syndrome, nephrotic syndrome, as well as the use of steroids or lipid-lowering drugs during the study period. The study was conducted according to the Nuremberg Code. All patients gave informed written consent.
consent, agreeing to all 10 points of the Nuremberg Code. The study was conducted at the Molecular Biology Laboratory for Genetics, Medical Biochemistry Department at the Faculty of Medicine, Assiut University.

Disease severity of RA. RA disease severity was surveyed by assessing radiographic injury on X-rays of the hands and feet of the subjects. Radiographs of the hands and feet were taken at standard (start of study) and annually thereafter. Radiographic severity was scored by two rheumatologists blinded to examine convention in sequential request for disintegrations and joint space narrowing as per the Sharp van der Heijde Score (SHS) strategy (17). Patients whose SHS expanded by in excess of five focuses (ΔSHS>5) from pattern to 1 y were considered to have fast radiographic movement. Patients whose SHS increased by more than five points (ΔSHS>5) from baseline to 1 y were considered to have rapid radiographic progression (17, 18).

Clinical and laboratory data. All patients and controls underwent complete clinical examination, abdominal ultrasound. Blood specimens were collected after overnight fasting and analysed for routine lab work (liver function tests using a BM Hitachi 711 Chemistry Analyzer), prothrombin time and concentration using a Bench Electronic coagulator, complete blood count (CBC) using a SYS-MEX K1000 device, hs CRP (Immunodiagnostic AG, USA), erythrocyte sedimentation rate (ESR) by Westergren’s method in mm/h and rheumatoid factor (RF).

From each participant, 10 mL fasting blood samples were obtained by venepuncture into tubes containing Na2EDTA or no anticoagulant. Care was taken to prevent any physical damage which might cause haemolysis. The tubes were allowed to stand at 4°C in room temperature for few minutes then centrifuged at 3,000 rpm (1,000 ×g) for 20 min at 4°C. The clear, non-haemolysed clarified supernatants serum or plasma were quickly removed and aliquoted into sterile 1 mL tubes and stored at −70°C until used.

Measurement of sRAGE, IL-6, IL-8, NGAL, oxLDL, HIF-1α and P3NP (P3NP; procollagen 3 N-terminal peptide). Plasma concentrations of sRAGE, IL-6, IL-8, NGAL, oxLDL, HIF-1α and P3NP were determined by enzyme-linked immunosorbent assays (ELISA) and following the instructions supplied with each kit.

Measurement of sRAGE: sRAGE levels in sera were determined by RAGE ELISA-based Immunoassay (Wkea Med Supplies Corp, New York, USA), with wells covered with murine anti-human RAGE immobilised antibody with which serum samples were incubated. A polyclonal capture antibody against the extracellular domain of P3NP was used for detection. After washing, streptavidin conjugated horseradish peroxidase (HRP) was added to the wells. The minimum detectable concentration of P3NP is 0.64 U/mL according to the manufacturer.

Measurement of IL-8: (RayBio® Human IL-8 ELISA kit Catalogue #: ELH-IL8). The minimum detectable IL-8 concentration is 20 pg/mL according to the manufacturer.

Measurement of NGAL: (Hycult Biotech CAT#HK 330-01, HNL, lipocalin 2, oncogene 24p3, LCN2, ELISA kit). Producer’s instructions were monitored. A 2,000-fold dilution was used to bring concentrations of each sample within the 100-fold sensitivity range of the assay (0.4–100 ng/mL). All diluted samples fell within the range of the calibration curve. The minimum discovery limit of the assay was 0.4 ng/mL. The intra-assay and inter-assay coefficient of variation was 1.1% and 3.2%, separately, at 65 ng/mL.

Measurement of oxidized LDL: (MDA-LDL, Human ELISA Kit abcam; ab242302). The discovery sensitivity limit was <15 ng/mL.

Measurement of HIF-1α: (CUSABIO, Code#CSB-E12112h). The smallest dose of human HIF-1α was determined to be 62.5 pg/mL, 4.000 pg/M and the sensitivity was 15.6 pg/mL.

Measurement of P3NP (P3NP; procollagen 3 N-terminal peptide): (MyobiosourceCAT#MBS282472Human P3NP). Following the instructions supplied with the kit, an antibody specific for P3NP was pre-coated onto a microplate. A polyclonal capture antibody against the extracellular domain of P3NP was used for detection. After washing, streptavidin conjugated horseradish peroxidase (HRP) was added to the wells. The minimum detectable concentration of P3NP is 0.64 U/mL according to the manufacturer.

Genomic DNA extraction and genotyping. Four milliliters of peripheral blood was collected EDTA-containing tubes and stored at −80°C until patch analysis. Genomic DNA was separated utilising the Wizard Genomic DNA Purification Kit obtained from Promega. After extraction, the accuracy and purity of the material was tested using agarose gel and a spectrophotometer. Two VDR SNPs were amplified using PCR and genotyped. Bsm1 (VDR 1544410 A>G) rs1544410 polymorphic site: forward: 5’CAACCAAGACTACAAGTACGCGCTCAGTGA3’ and reverse: 5’AACACCGGGGAGGTCAGGGG3’. FokI (VDR 2228570 C>G) rs2228570 polymorphic site: forward: 5’AGCTGGCCCTGGCACTGACTCTGCTCT3’ and reverse: 5’ATGGCAACCTTGGCTCTTTTCCTCCTC3’. Genotyping of Bsm1 (VDR 1544410 A>G) and FokI (VDR 2228570 C>T) polymorphisms in the VDR gene was performed using PCR–RFLP. Enhancement responses were set up independently for FokI and Bsm1 polymorphic locales of VDR quality utilising primers given by Harris et al. (19) and Morrison et al. (20). The PCR was performed using Taq PCR Master Mix pack (Qiagen, GMBH) as following: 25 μL of Taq PCR master mix blend was added into each PCR cylinder, followed by the addition of 100 ng of extricated DNA, 25 mM forward primer, and 25 mM reverse primer (Operon Biotechnologies, Inc.) and double-distilled water (ddH2O) was finally added to bring the entire volume to 50 μL. Following denaturation at 94°C for 5 min, amplification was performed for 35 cycles with denaturation at 94°C for 30 s, annealing at
63˚C for 30 s for BsmI SNP and FokI at 58˚C for 30 s and expansion at 72˚C for 30 s. The last expansion was permitted to continue at 72˚C for 5 min; 8 mL of the PCR mix was processed overnight with 10 U BsmI at 37˚C and for 3 h with 10 U FokI at 65˚C (MBI, Fermentas, Lithuania). The PCR products were visualised on a 3% agarose gel stained with ethidium bromide.

**Statistical analysis.** Data analysis was carried out using IBM-SPSS/PC/VER 21. Descriptive statistics: quantitative variables were presented in terms of mean±standard deviation (SD), median while qualitative variables were expressed as frequencies and percentages. Test of normality (Kolmogorov-Smirnov) was conducted to determine the type of distribution of data. Chi-square/Fisher exact test was used to assess differences between two proportions. Student t-test/Mann-Whitney U test was used to test the mean/median differences of the data according to its normality. Correlation analysis was used to test the association between variables (Spearman’s rank correlation). ROC curve was depicted to explore the predictive performance of biochemical indices (NGAL, oxLDL, sRAGE, HIF-1α, HsCRP, IL-6, IL-8 and P3NP) for RA severity (high severity), analysed as area under the curve (AUC), standard error (SE) and 95% CI. The probability of less than 0.05 was used as a cut off point for all significant tests. Multivariate logistic regression analysis was used to investigate the predictive power of the genotype and alleles of the studied cohort on the RA severity categories (odds ratio (OR), 95% confidence interval (95% CI) and likelihood ratio test-LRT).

**Ethical considerations.** Approval for this study was obtained from Institutional review board (IRB) of Faculty of Medicine, Assiut University in line with the declaration of Helsinki guidelines (IRB number: 17200361) prior to study execution. In addition, all participants received a written consent form. The informed consent was clear and indicated the purpose of the study, and their freedom to participate or withdraw at any time without any obligation. Furthermore, participants’ confidentiality and anonymity were assured by assigning each participant with a code number for the purpose of analysis only. The study was not based on any incentives or rewards for the participants.

**RESULTS**

**Baseline characteristics of participants**

Table 1 showed the socio-demographic characteristics of the studied patients. The patients with RA were significantly older (45.7±11.6 y) than controls (35.9±11.9 y). Females represented 90% of the cases and 95% of the controls. The median values of all biomarkers were statistically significantly higher in RA cases than in controls. There was significant difference in distribution of the FokIrs2228570FF genotype between cases and healthy controls.

**RA predictors among the study cohort**

After adjusting for age and sex, the final regression
The model included six predictors: ESR, RF, oxLDL, sRAGE, IL-6, and P3NP. With one-point increase in the levels of ESR and RF, the increase in the probability of getting RA was 31% and 1%, respectively. Moreover, with 1 IU/mL increase in the oxLDL, there was an increase in the prediction of RA by 37%. Likewise, with 1 ng/mL increase in the sRAGE, there was an increase in the prediction of RA by 1%. Notably, there was a 4-fold increase in RA affliction risk with 1 pg/mL increase in the levels of IL-6 and IL-8. Lastly, the risk of getting RA was 2.6 times higher with every mg/L increase in the P3NP level (Table 2).

### Table 2. Independent predictors of RA in multivariate logistic regression analysis.

| Factor            | Odds ratio | 95% CI | p-value |
|-------------------|------------|--------|---------|
| Age/y             | 1.097      | 1.039–1.167 | =0.003  |
| Sex (Male)        | 1.673      | 0.436–6.425 | =0.453  |
| ESR               | 1.311      | 1.189–1.445 | <0.001  |
| RF                | 1.012      | 1.006–1.018 | <0.001  |
| NGAL ng/mL        | 1.516      | 0.001–1273 | =0.972  |
| oxLDL IU/mL       | 1.368      | 1.244–1.504 | <0.001  |
| sRAGE ng/mL       | 1.009      | 1.006–1.012 | <0.001  |
| HIF-1α ng/mL      | 1.016      | 0.001–3284 | =0.980  |
| IL-6 pg/mL        | 4.148      | 1.982–10.095 | <0.001  |
| IL-8 pg/mL        | 1.168      | 0.001–812.8 | =0.993  |
| P3NP μg/L         | 2.637      | 1.956–3.555 | <0.001  |
| FokIrs2228570FF (CT) | 1.885   | 0.784–4.530 | =0.157  |

1 CI, confidence interval. IL-6, interleukin-6; IL-8, interleukin-8; HIF-α, hypoxia inducible factor-alpha; sRAGE, soluble receptor of advanced glycation end product; oxLDL, oxidized low-density lipoprotein; NGAL, neutrophil gelatinase-associated lipocalin; P3NP, procollagen N-propeptide of type III collagen.

There was positive moderate correlation between NGAL and oxLDL (0.45, p<0.001). Furthermore, there was positive high correlation between sRAGE and oxLDL (0.7, p<0.001). Moreover, there were negative mild correlations among IL-6, HIF, and oxLDL (−0.19, −0.29; p<0.05). On the other hand, there were positive mild correlations among P3NP, sRAGE and oxLDL (0.28, 0.31; p<0.05) (Fig. 2).

### Baseline characteristics of the studied RA cases and difference in biomarker levels according to disease severity

The study cases were categorised according to disease severity into two groups: remission/low activity/moderate activity and high activity. There were no statistically significant differences in the median values of the biomarkers between the two groups (p>0.05).

### Diagnostic performance of biochemical indices for RA severity prediction

The predictive accuracy of the models for NGAL and IL-6 were acceptable (AUC=0.631 and =0.622, respectively, p<0.05). However, the models for the other biomarkers had AUC of 0.5 or less, thus were not acceptable (Fig. 3).

### Genotypic and allelic frequencies of the RA severity groups of FokIrs2228570FF and BsmIrs1544410

It was found that in high activity cases, frequencies of ‘CC’ and ‘CT’ were higher than those in low activity cases for the FokIrs2228570FF polymorphic site. These differences were statistically insignificant (p>0.05). For BsmIrs1544410, ‘GG’ and ‘AG’ genotypes were significantly associated with high RA activity (OR: 3.15, 95% CI 1.10–6.25 and 2.27, 95% CI 1.02–5.63, p<0.05). Likewise, the frequency of genotypes ‘AG+GG’ was higher in high activity cases than in low activity cases for the BsmIrs1544410 polymorphic site (OR: 3.03, 95% CI 1.12–6.99, p<0.05) whereas G-allele frequency was higher in high activity cases than in low activity cases on the BsmIrs1544410 polymorphic site (OR: 2.30, 95% CI 1.03–8.01, p<0.05) (Table 3).

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**Fig. 2.** Correlation between NGAL, oxLDL, sRAGE and HIF-1α in RA cases. HIF-1α, hypoxia inducible factor-alpha; sRAGE, soluble receptor for advanced glycation end products.
Levels of different markers according to FokIrs2228570FF (C/T) (A/G) and BsmIrs1544410 (A/G) genotypes

It was found that the serum levels of NGAL and IL-6 were significantly different according to FokIrs2228570 (C/T) genotypes \( (p<0.05) \), whereas according to BsmIrs1544410 (A/G) genotypes it was found that the serum levels of s-RAGE and PN3P were significantly different \( (p<0.01) \) and the serum levels of oxLDL was \( (p<0.05) \).

**DISCUSSION**

The pathogenesis of RA is multifaceted and involves numerous features. Interaction between the environment and genetics is important at all stages of the disease. Vitamin D plays a major role in RA development (21). Previous studies have reported that lower levels of vitamin D are widespread among the patients with RA, affecting up to 65% of them (17, 18).

Our study presented RA cases with BsmIrs1544410, ‘GG,’ and ‘AG’ genotypes, which were significantly associated with high RA activity, and the frequency of genotypes ‘AG+GG’ and the G-allele was higher in high activity cases than in low activity cases. Regarding the FokIrs2228570FF polymorphic site in high activity cases, the frequencies of ‘CC’ and ‘CT’ were more prevalent than those in low activity cases. Thus, our current study demonstrated the strong association of VDR gene polymorphism (rs2228570 and rs1544410) with the onset of RA in the studied population. Numerous studies have also testified noteworthy association of VDR...
gene polymorphism and RA (19, 20, 22, 23). Nonetheless, no noteworthy affiliation was accounted for in other countries (20, 22–26).

Garcia-Lozano et al. (27) suggested that polymorphisms in the VDR gene could have some effects on RA, affecting the disease onset. Therefore, it is conceivable that polymorphic genes whose products have a direct effect on calcium/vitamin D metabolism may play a role in the pathogenesis. Additionally, Provedini et al. (28) and Bhalla et al. (29) discovered that the detection of VDR polymorphisms in monocytes and activated lymphocytes confirms a role in immunoregulation and joint inflammation.

Studies on the genetic background of patients with RA provide the first indication that VDR polymorphisms are linked to RA. BsmI polymorphism of VDR gene is involved in the pathogenesis of osteoporosis in RA (28), and the FokI polymorphism of VDR is associated with RA in Europeans (29, 30). Previous studies have demonstrated an increased transcription rate (1.7-fold) of the VDR gene in cells (24, 31). Gao and Yu (32) reported that VDR rs731236, rs1544410, rs2228570, and rs7975232 polymorphisms were significantly associated with autoimmune thyroid diseases (AITD) risk. Salimi et al. (33) showed that the VDR rs2228570 and rs731236 polymorphisms were associated with systemic lupus erythematosus (SLE) risk. Others (34) observed that there is a significant relationship between VDR gene, Apal, BsmI polymorphisms, and urinary tract infection (UTI) in children. The results indicate that these polymorphisms may play a role in pathogenesis of UTI.

Moreover, Rass et al. showed that women with RA with the VDR TaqI TT genotype had faster bone loss during the three years of study compared with women with the VDR TaqI TT genotype. The BsmI genotype was not associated with any markers of disease activity: the VDR BsmI B allele was associated with higher titres of the RF. These results suggest that the B allele may be a marker for augmented osteoclastic activity in patients with RA. These studies recommended that the VDR FokI polymorphism could be an indicator for familial RA (35).

To our knowledge, the association between inflammatory biomarkers and antioxidants indices has not been assessed previously.

Our study found that with each 1 ng/mL increase in the sRAGE, there was increase in the prediction of RA affiliation by 1%. Jafari Nakhjavani et al. (36) found higher serum sRAGE levels in patients with RA than in healthy controls, which was positively correlated with disease activity. Others observed an association of low sRAGE levels and RAGE 82Ser polymorphism, which is found more frequently in patients with RA. sRAGE and S100 proteins were related with RA inflammation and autoantibody creation resulting in end-organ impairment (37). This study concluded that RAGE action impacts co-improvement of joint and vascular infection in RA.

Notably, our study found that there was an increase in the RA affiliation risk with an increase in the levels of IL-6 and IL-8. This was similar to the finding of Sattar et al. (38) who found a significant difference in the levels of IL-6 in BsmI genotypes: patients with the Bb genotype had a higher median level of IL-6 than patients with the BB and bb genotypes. They concluded that the pro-inflammatory cytokines, generated in the synovial fluid, can be released into the systemic circulation (39, 40).

Conversely, our study showed positive correlations among P3NP, sRAGE and ox-LDL (p<0.05). We also found that the serum level of P3NP was significantly higher in RA compared to healthy controls. However P3NP levels showed no important association either to disease activity or to the severity. These results agree with the findings of, Tebib et al. (9) who also demonstrated that there was a durable link between X-ray grade and P3NP standards. Besides, Sharif et al. (10) suggested that synovial fluid P3NP concentrations might reveal limited synovial proliferative processes in joint disease, and that they could be of scientific and predictive value in inflammatory arthropathies.

Kishimoto found that the serum levels of IL-6 were higher in the patients with RA (41). IL-6 also plays a role in angiogenesis by encouraging intracellular adhesion molecules. These roles of IL-6 in the pathogenesis of RA make IL-6 a remarkable target for RA treatment (40).

Our study further revealed that the LCN-2 levels were higher in patients with RA than in healthy subjects, which results from joint damage. Previous studies have shown the role of neutrophil impairment in the etiopathogenesis of RA (42). In neutrophils, LCN-2 expression is related to the initiation of inflammation (43, 44). In our investigation, NGAL levels were higher in the patients with RA than in the healthy controls. Katano et al. (44) established in their examination that serum NGAL levels were higher in patients with RA than in the osteoarthritis patients and healthy controls. Based on our findings, we can conclude that the serum NGAL levels in patients with RA are not directly correlated with inflammatory activation, which shows a possible association with joint damage (45, 46).

Our study also found that the median values of HIF-1α were significantly higher in RA cases than in controls. Ajeganova et al. (47) reported higher oxLDL concentrations in patients with RA who experienced subsequent cardiovascular disease than healthy controls. Previous studies suggest that the oxLDL/LOX-1 system plays a vital role in the development of RA (48, 49). These observations support the hypothesis that hypercholesterolemia is a risk factor of arthritis, and oxLDL is involved in cartilage matrix degradation in RA. Others reported that hypoxia and HIFs are involved in significant pathophysiological characteristics of RA, with cartilage destruction. HIF-1α is very hard to detect under normal condition, but it is highly expressed in hypoxic or inflammatory conditions in a variety of cells (47, 49).

In addition, Brouwer et al. (50) established that
HIF-1α expression is the strongest in the sub-lining layer of RA synovium and is interrelated to both angiogenesis and inflammation in the synovium from patients with RA. They concluded that HIF-1α could serve as a vital new therapeutic target in RA.

CONCLUSIONS

These outcomes taken together support the immunoregulatory role of vitamin D mediated through VDR, inflammatory, angiogenic biomarkers, and bone loss in RA. The difference between our results and the results of other studies is the genetic polymorphisms of VDR, which indicates that vitamin D receptor polymorphism is associated with several inflammatory diseases. However, the present study has some limitations that must be observed, such as the cross-sectional design, which did not allow to create connection between the conclusions realized. Besides, the genetic associations and the results observed in this study need replication in larger cohorts. As For a genetic susceptibility study, a prior sample size calculation based on SNP frequency is a requisite. Importantly, studies of this kind are no usually powered with 100 individuals, which represent a major limitation. Thus studies with larger sample sizes that show deliberate gene-gene and gene-environment interactions are desirable to confirm our results. And it proves this field worthy of further research.

Authorship

Naglaa K Idriss: designing concept, conceptualization, methodology, laboratory and genetic testing of the biomarkers, investigation, writing up and visualization. Zahraa I Selim: conceptualization, validation, supervision. Eman H El-Hakeem: acquisition of data; analysis and interpretation. Fatma H El Nouby: drafting the article and revising it. Ahmed K Ibrahim: methodology, software, writing–formal analysis. Hayam G Sayyed: editing of manuscript. Dalia A Elgamal: editing of the original draft, critical review and submission. Maggy A Ibrahim: revising it critically and analysing. Doaa Kamal: interpretation of data and drafting the work. Samar H Goma: conceptualization, methodology, investigation, writing–review and editing.

Disclosure of state of COI

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