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Original Research

Evaluation of vitamin D3 level, anti-CCP and some inflammatory markers in women with rheumatoid arthritis in Al-Hawija district, Kirkuk governorate

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Abstract:

The present study aimed to know the role of rheumatoid arthritis on the level of Anti-Cyclic citrullinated peptide (ACCP), C-Reactive protein (C-RP), Erythrocyte sedimentation Rate (ESR), and vitamin D3 in patients with rheumatoid arthritis in Al-Hawija district, Kirkuk governorate. The experiment included 50 samples, 25 of which were infected with this disease, which is the second group, and 25 healthy, which is the first group. The ages of the experiment samples ranged between 50-60 years. After ensuring that they were free from other chronic diseases, the samples were collected. "The results of the current study revealed a significant increase (P≤0.05) in the level of ACCP in patients compared to healthy women, while the results of the current study indicated a significant increase (P≤0.05) in the level of C-RP in patients compared to healthy women. The current study also revealed a substantial (P≤0.05) increase in the patients' ESR level when compared to healthy women, as well as a significant (P≤0.05) drop in the patients' vitamin D3 level when compared to healthy women. It can be concluded from the results of the current study that those infected are exposed to immunodeficiency and the disease may cause osteoporosis due to the low level of vitamin D3".

Keywords: "Rheumatoid arthritis; Anti-Cyclic citrullinated peptide; C-Reactive protein; Erythrocyte sedimentation Rate"

1-Introduction:

Rheumatoid arthritis (RA) is a highly widespread immune-mediated inflammatory illness that often affects small and big joints symmetrically, causing pain, edema, and stiffness in its early stages,Chronic inflammation of the synovium, the lining that lines the joints, causes severe joint

damage, disability, and loss of productivity if therapy is not received, The treatment of this illness has undergone a revolution over the previous 25 years, leading to significantly greater rates of disease remission and improved long-term results, This progress is a result of a paradigm change toward early and aggressive pharmacological intervention together with a multiplicity of treatment options, which in turn are linked to improved pathobiological knowledge and the introduction of novel rheumatoid arthritis medications, After providing a general audience with an outline of these advancements historically, this review concentrates on more recent, focused treatments in a constantly changing field [1]. Women are affected by RA two to three times more frequently than men, with a prevalence of roughly 1%. Individuals aged 35 to 55 are the most commonly affected. Although the precise and primary causes of RA are unknown, it is generally accepted that a person's genetic background, environmental variables, and epigenetic markers all operate as risk factors for the illness. It is widely acknowledged as a notion that RA arises from a high-risk genetic background that generates novel epitopes when it combines with epigenetic markers and environmental variables. It sets off a series of events that eventually result in the development of inflammatory cytokines and synoviocytes, which causes destructive and chronic arthritis [2]. The interaction of fibroblast-like synoviocytes (FLSs), which line the synovial membrane, The clinical symptoms that individuals with RA experience in their joints are caused by a combination of cells from the "innate immune system (macrophages, dendritic cells (DCs), natural killer (NK) cells, mast cells, and neutrophils) and the adaptive immune system (B and T lymphocytes; B- and T cells). These cells express toll-like receptors (TLRs), which are engaged in many responses related to tissue injury" [3]. As RA cell participants, FLSs are thought to be affected by immune system-secreted cytokines and undergo a quiescent phase that is followed by an invasive and hyperplastic character akin to tumor cells inside their unique milieu. Gene expression patterns in rheumatoid ligament synovitis indicate that healthy people do not express the related genes. Angiogenesis factors, proteases, and several cytokines are produced by these cells, which ultimately cause the afflicted joints to become severely inflamed [3]. The preclinical phases of seropositive rheumatoid arthritis are distinguished by the production of ACPAs both locally and systemically, as well as by impaired immunity, which is frequently linked to mucosal surfaces such as those of the gastrointestinal tract, lungs, and oral cavity. The blood can show these autoantibodies 4.5 years in advance of the onset of arthritis on average [4]. Autoantibody levels rise over time, increasing the risk of rheumatoid arthritis. As this preclinical stage goes on, proinflammatory proteins in the blood increase and ACPAs targeted against a growing range of protein epitopes follow, eventually leading to joint inflammation [5]. The identification of neoantigens can be induced by a variety of protein changes, such as carbamylation, malondialdehyde–acetaldehyde adduct formation, and others, leading to the development of antibodies against these transformed protein antigens. The immune system's reaction to modified peptides is not limited to citrullination [6]. though there's also a good chance that other sex-related characteristics have a role. Numerous infectious pathogens, such as the In addition to bacteria like oral Porphyromonas gingivalis and intestinal prevotella species, viruses such as Epstein-Barr virus, retroviruses, bacterial superantigens, and mycoplasma species , have been suggested as etiologic or contributory factors [7]. It seems improbable that a single microbe causes all of the illness, though. The most prevalent behavioural risk factor for the development of rheumatoid arthritis is cigarette smoking . Other factors that somewhat raise the risk of rheumatoid arthritis are using oral contraceptives, being obese, and having poor vitamin D levels. A Mediterranean diet, the consumption of omega-3 fatty acids, supplementing with fish oil, and alcohol consumption are factors that reduce the risk [8].

Genetics is the main risk factor for rheumatoid arthritis. The chance of developing rheumatoid arthritis increases by a factor of two to five for first-degree relatives of affected individuals. Among genetic associations, the most significant is the HLA-DR locus,The "shared epitope," or Amino acids 70–74, which are well-characterized locations in the hypervariable region of the HLA-DRβ chain, are associated with an increased risk. Because HLA-DR has the ability to bind and display certain arthritogenic peptides, may enhance susceptibility when it comes to antigen presentation to CD4+ T cells, Citrullinated peptides can bind to HLA-DR genes linked to rheumatoid arthritis more avidly than native peptides, which can activate T cells and produce cytokines [9]. The severity of rheumatoid arthritis and an individual's susceptibility to the disease are largely determined by environmental and behavioral factors. Genetic risk and cigarette smoking can work in concert. For example, the risk of rheumatoid arthritis is 20 times higher among smokers who test positive for ACPA and have two copies of the susceptibility shared epitope than in nonsmokers [10]. Following quitting smoking, the risk decreases steadily until it reaches the danger in 20 to 30 years for people who don't smoke [11]. In those who carry rheumatoid arthritis risk alleles, environmental exposures like cigarette smoke can cause inflammation and stress at mucosal surfaces, which can lead to the onset of the disease. The largest correlation has been shown between mucosal inflammation and rheumatoid arthritis in the respiratory system. Peptidyl arginine deiminases are enzymes found in the mammalian genome that convert arginine into citrulline. Numerous proteins are post-translationally citrullinated as a result of these enzymes, which are brought on by cellular stress. Smokers' airways are very active in the process of citrullination, and macrophages have been found to have altered peptides [12].

2- Materal and methods:

2-1 Study population:

After completing an initial physical examination that demonstrated they were free of known chronic conditions, women from the Al-Hawija district in the Kirkuk Governorate, aged 50 to 60, participated in the study. Two groups of women participated in the study: those without rheumatoid arthritis and those with the condition.

2-2 Blood collection and biochemical measurements:

Both the controls and the patients had blood drawn. Two portions of the separated serum were separated. The first component was utilized immediately (fresh) to measure CRP and ESR using latex agglutination test kits that were supplied by CRP: Biokit, Spain, and the UK. The other portion was refrigerated until anti-CCP was detected using ELISA kits (IMMUN Co., DIAGNOSTICS/ Buffallo /NY/Cat No.8001). Vitamin D status was assessed by ELISA.

2-3 Statistical Analysis

"The Statistical Package for the Social Sciences (SPSS 12.0 for Windows, SPSS Inc. Headquarters, Chicago, USA) was used to conduct the statistical analysis. The values were presented in the format of mean ± SD. The paired samples t-test was used to assess differences between different time points within each group, and the independent samples t-test was used to compare groups. P-values below 0.05 were regarded as significant differences"[13].

3-Results:

the chance in two to three days for nonsmokersThe study's findings showed statistically significant differences between the groups: women with rheumatoid arthritis had a significantly higher level of ACCP (28.85±2.99) than women without the illness (2.34±0.28), with a significant rise (P≤0.05). Although the study's findings showed statistically significant differences, women with rheumatoid arthritis had significantly higher levels of CRP (12.57±0.99) compared to those without the condition (4.73±0.34) over the course of several years. The present study's findings demonstrated statistically significant differences, with women with rheumatoid arthritis showing a significantly higher level of ESR (36.28±3.65) compared to those

without the illness (11.36±1.38). The current study's findings showed that women with rheumatoid arthritis had significantly lower levels of vitamin D3 (15.24±1.30) than women without the condition (P≤0.05) (39.6±3.32) showing in Table (1)

.Table (1) shows the vital and inflammatory values of patients with rheumatoid arthritis.

Parameters/ Groups	ACCP (EU/ml)	CRP (mg/L)	ESR (mm/hr)	Vit.D3 (nmol/L)
Control (n=25)	2.34±0.28 b	4.73±0.34 b	11.36±1.38 b	39.6±3.32 a
Patients (n=25)	28.85±2.99 a	12.57±0.99 a	36.28±3.65 a	15.24±1.30 b

The different letters vertically indicate the presence of significant differences between patients and healthy individuals at a probability level of (p≤ 0.05)

4- Discussion:

The results of the current investigation demonstrated a substantial drop (P≤0.05) in the level of vitamin D3 in patients compared to healthy women. Previous research findings indicate that serum vitamin D levels are lower in RA patients than in showing RA disease activity is negatively correlated with blood vitamin D levels in healthy subjects in RA patients. Following two independent investigations that found a substantial inverse relationship between serum vitamin D3 concentrations in patients with active RA, this meta-analysis was conducted [14]. Vitamin D has been shown to have effects on physiological systems that go well beyond its well-known functions in preserving bone and calcium homeostasis. Among these are the potent immune-suppressive characteristics of the active form of vitamin D, 1,25-dihydroxyvitamin D3 (1,25-(OH)2D3). Furthermore, it has been demonstrated that antigen-presenting cells produce 1α-hydroxylase, an enzyme that facilitates the conversion of precursor 25-hydroxyvitamin D3 (25-OHD3) into 1,25-(OH)2D3, which in turn allows vitamin D to be absorbed and activated by immune microenvironments. Due to this intracrine mechanism at the local level, immune responses might vary based on the availability of 25-OHD3, and autoimmune disorders such as rheumatoid arthritis have been linked to vitamin D deficiency. [15]. According to Franco et al. [16], vitamin D deficiency in RA patients is only clinically significant if vitamin D supplementation is expected to alleviate symptoms of the disease or even stop RA in individuals who are at risk of developing the condition. The number of patients, features, length and severity of the disease, concurrent treatment plan, kind and duration of the supplementing regimen, and number of outcomes in vitamin D supplementation trials for RA have all varied significantly to far. Nine randomized controlled trials of vitamin D supplementation for at least three months in rheumatology were found by a recent meta-analysis, five of which included RA patients.

The current study's findings demonstrated that patients' levels of ACCP were significantly higher (P≤0.05) than healthy women. the European League Against Rheumatism (EULAR) and the American College of Rheumatology ACCP defined RA as having synovitis in at least one joint, even in the absence of a more appropriate diagnosis, and receiving a total score of six or higher out of ten in four distinct areas [17]. Autoantibodies, or ACCP, are produced when citrullination is dysregulated and are a component of the immune response [18]. demonstrated the existence of ACPA, since the lingering protein in

the circulation or joint promoted the immune complex's creation, causing injury and swelling in the joint [19]. Its sensitivity ranges from 41–66% for early RA and 41–77% forestablished RA, while its specificity ranges from 88– 98% , Furthermore, over 80% of RA patients exhibit elevated levels of ACCP antibodies [20]. The precise cause of this is unknown, however some theories include humoral reactions to bacteria that may trigger the inflammatory process that leads to the development of RA and the possibility that various microorganisms produce arginine to citrulline deiminase activities [21]. by stimulating the production of T cells and then B cells in response to citrullinated antigens by the immune system. Nonetheless, numerous investigations have replicated the anti-CCP titer's long-term stability [22]. In RA patients, MRI bone erosion scores and ACCP positivity are correlated with early identification of RA, which raises It [23]. while The present study's findings indicated a noteworthy rise ($P \leq 0.05$) in the degree of C-RP in patients compared to healthy women. There are also many other vital signs that show how rheumatoid arthritis begins, including: CRP [24]. The conventional methods for diagnosing RA disease include serological inflammatory testing, particularly CRP. These tests are widely accessible, reasonably priced, and useful as regular diagnostic tools for inflammatory illnesses as well as follow-up measures to track the effectiveness of treatment. They support the clinical symptoms and indications of inflammation and give doctors useful information [25]. Elevated CRP levels, together with joint count assessments, can suggest the existence of an active inflammatory response. Many autoantibodies are involved in the disease pathogenesis due to an autoimmune mechanism and contribute in the diagnosis and prediction of RA [26]. Hu et al [27] observed elevated amounts of RA sera and synovial fluids, which they connected with CRP readings. It contributes to the induction of synovial fibroblasts in addition to causing inflammation. Fadda et al found that when RA patients were compared to controls, their serum MMP-3 levels were greater. Patients with RA who had elevated CRP and ACCP sera had significantly greater amounts of these substances. A noteworthy correlation is reported between MMP-3, a marker of disease activity. Additionally, there was a noticeable distinction between individuals who had erosions and those who did not. Ra patients' high MMP-3 serum levels are a particular sign for joint destruction and an indicator of the disease activity of the patients [28]. Additionally, the current study's results demonstrated a substantial increase ($P \leq 0.05$) in the ESR level. in patients compared to healthy women. Another biomarker that shows how rheumatoid arthritis starts is ESR [24]. Using a large cohort of patients who reflected rheumatoid disease in the clinic, we studied the target tissues of rheumatoid arthritis and found a strong significant link between erythrocyte sedimentation rate and histological inflammatory alterations in synovial biopsies [29]. Additionally, the discovery that women often had higher ESRs than men does not conflict with the findings of earlier research [30]. Similar to sex differences, specific immunological or haematological alterations, such as hormonal changes in menopausal women , may account for the rising ESR levels with aging [31].

5- conclusion:

Our study's findings support the notion that women with rheumatoid arthritis have reduced vitamin D3 levels . The results also indicate that inflammatory mediators in the study samples (ACCP, ESR and CRP) were very high, which increases the risk of developing other immune diseases. It also indicates a possible relationship between osteoporosis and rheumatoid arthritis due to low vitamin D3. Further research More research in other nations is required to verify whether or not these findings are consistent.

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