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Levels of Interleukin 33 and Interleukin 27 in Rheumatoid Arthritis and Osteoarthritis Egyptian patients

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ABSTRACT

Background: - Cytokines are important mediators of immune functions in humans and animals. IL-27, an IL-12 family cytokine, exerts a critical role in immune regulation in the context of infection and autoimmunity. IL-33, a newly foundIL-1 family cytokine is involved in joint inflammation in rheumatoid arthritis (RA). So, we aimed to investigate the immunopathological roles of IL-27 and IL-33 in serum and synovial fluid (SF) of RA versus osteoarthritis (OA) patients.

Patients& Methods: - This study was conducted on a total number of 69 individuals, 31 ofthem were RA (22 females, 9 males) and 23 patients were OA (15females, 8 males), and 15 control blood donors (10 females, 5 males).All subjects were evaluated bymeasuring CBC, ESR, high-sensitivity CRP (hsCRP), RF, IL-27 and IL-33 in the bloodwith measurement of hsCRP, RF, IL-27 and IL-33 in the SF of 20 RA and 18 OA patients.

Results: - The mean WBCs,ESR,serum CRPand RF (RF-IgM) in addition to thedetection percentages of both serum IL-27 and IL-33 were significantly higher in RA than OA and control groups, also,the mean of both SF CRP and RF,as well as the detection percentages ofboth SF IL-27 and IL-33were significantly higher in RA than OA patients. While, there was no significant difference between serum and SF CRP, RF, IL-27 or IL-33 in RA andOA groups.In RA group, bothserum IL-27and IL-33 showed significantly positive correlations withDAS28 while the former has also with CRP and the later has also with serum RF.

Conclusion:-IL-33 and IL-27 played an important proinflammatory role in the pathogenesis of RA. Considering their correlation with disease activity, they may become potentialtherapeutic targets for RA.

Keywords: Rheumatoid Arthritis, Osteoarthritis, Interleukin-27 and Interleukin-33

INTRODUCTION:

RA is the most common connective tissue disease affecting approximately 1% of the population and represents an increasing burden on global health resources. Survival in RA patients is lower than that of the normal population *[36]*. RA is a chronic, usually progressive, systemic inflammatory condition of unknown cause. It is characterized by synovial proliferation and a symmetric, erosive arthritis of peripheral joints. The hallmark feature of the disease is persistent symmetric polyarthritis (synovitis) that affects the hands, wrists and feet,

although almost all diarthrodial joints may become involved. In addition to articular manifestations, systemic involvement may cause constitutional symptoms; rheumatoid nodules, serositis and vasculitis. The severity of RA may fluctuate over time, but chronic RA most commonly results in the progressive development of various degrees of joint destruction, deformity, significant decline in functional status and a premature death *[37]*. Osteoarthritis (OA) is a degenerative disease, a consequence of agerelatedchanges, genetic predisposition and abnormal biomechanicalforces that lead to an alteration of metabolic processes and destruction ofarticular cartilage. The osteoarthritis disease process affects not only thecartilage but also the entire joint structure, including the synovialmembrane, bone, ligaments and periarticular muscles *[2]*.Cytokines which comprise of a family of proteins act in concert with specific cytokine inhibitors and soluble cytokine receptors to regulate the human immune response. An imbalance in cytokine production or cytokine receptor expression and/or dysregulation of a cytokine process contributes to various pathological disorders *[48]*. IL-27, an IL-12 family cytokine, exerts a critical role in immune regulation in the context of infection and autoimmunity. IL-27 is composed of Epstein–Barr virus-induced gene 3 (EBI3), which is homologous to IL-12p40, a unique IL-12p35-like protein, and p28. IL-27 receptors are expressed in T cells, natural killer (NK) cells, monocytes, mast cells, activated B cells, and activated dendritic cells, and IL-27 is produced by antigen presenting cells. IL-27 receptor binding can activate STAT1 and promotes T-helper (Th) 1cell proliferation and interferon (IFN)-γ production. In addition, IL-27 directly promotes macrophage and mast cell activation to produce IL-1β and TNF-α *[10]*.IL-33, a member of the IL-1 family, is a ligand for the orphan receptor ST2 (also known as IL-1RL1). When IL-33 binds to ST2, it enhances inflammatory cytokines via the activation of nuclear factor-κB (NF-κB) and MAP kinases. Although it was initially thought that IL-33 was crucial for Th2 cytokine-mediated immune responses, it is now known that, it can overcome to have a role in RA *[53]*.

AIM OF THE WORK:

This study aims to investigate the immunopathological roles of inflammatory cytokines (IL-27 and IL-33) in RA by estimating serum and SF levels of IL-27 and IL-33 in RA patients and comparing them to OA patients and normal controls.

PATIENTS & METHODS:

A total of 69 individuals consisted of 31 patients with the diagnosis ofRA, 23 patients with the diagnosis OA and 15 control blood donors arecollected for this study.The study population was selected consecutively among patientswho presented to the outpatient Rheumatology clinics of Al-Hussein andSayedGalal hospitals. The RA group comprised 31 patients of age ranged from 17-65years. They were 22 females and 9 males. Their disease duration rangedfrom 4 months - 25 years. Diagnosis of RA was based the ACR revisedcriteria *[9]*and confirmed by ACR/EULAR 2010 criteria *[4]*. The OA group comprised23 patients of age ranged from 42-72years. They were 15 females and 8 males. Their disease duration rangedfrom 1-15 years. Patients with OA were diagnosed according to criteria for diagnosisof OA by the ACR *[6]*. The control group comprised 15 volunteers whose age ranged from27 to 53 years. There were 10 females and 5 males. All patients were subjected to complete history, full clinicalexamination with special attention to musculoskeletal system, andlaboratory investigations.For the RA group, patient assessment of pain was measured byvisual analogue scale (VAS), patient assessment of function wasmeasured by health assessment questionnaire (HAQ) and disease activitywas measured by disease activity score 28 (DAS28).For the OA group, patient's assessment for pain was measured byVAS and patient assessment of function (disability assessment) wasmeasured by the Western Ontario and McMaster Universities index(WOMAC index). They all had their

preferable blood sampled for CBC and ESR. The sera and synovial fluids of patients were collected for hsCRP, RF, IL-27 and IL-33 by ELISA kit (eBioscience).

Graph Pad Prism program version 5.0 was used for analysis of data. Data were summarized as mean ± SD. One-way analysis of variance was used for analysis of more than two variables, followed by the Turkey's post-hoc test for the detection of significance. Simple linear correlation (Pearson's correlation) was also carried out. P-value of up to 0.05 was considered significant, *[8]* Oxford: Blackwell Science.

RESULTS:

As shown in table 1, the mean of WBCs,ESR, serum hsCRP and serum RF were significantly higher in RA group than OA and control groups, the detection percentages of IL-27 and IL-33 in serum were significantly higher in RA than OA and control groups.

Table 1: WBCs, ESR, serum hsCRP and serum RF in RA, OA and control groups

Groups **Variables**	Control **N = 15**	OA **N = 23**	RA **N = 31**	P-value
WBCs (K/µl)	5.7 ± 2.3	6.7 ± 3.1	8.8 ± 4.1	0.011
ESR (mm/hr)	10.5 ± 5.2	21.8 ± 16.6	77.1 ± 43.8	< 0.0001
Serum hsCRP (mg/L)	3.8 ± 2	4.7 ± 3	14.7 ± 12.8	< 0.0001
Serum RF(IU/ml)	3.5 ± 1.9	5.3 ± 2.4	26.5 ± 28.6	< 0.0001
+ve Serum IL-27(pg/ml)	2(13.33%)	2 (8.7%)	14 (45.16%)	0.0047
+ve Serum IL-33(pg/ml)	1 (6.67%)	1 (4.35%)	12 (38.71%)	0.0027

As shown in table 2, the mean of SF hsCRP and SF RF were significantly higher in RA group than OA group. The detection percentages of IL-27 and IL-33 in SF were significantly higher in RA group than OA group.

Table 2: SF levels of hsCRP and RF in RA and OA groups

Groups Variables	OA N = 18	RA N = 20	P-value
SF hsCRP (mg/L)	4.9 ± 4.13	16.56 ± 15.35	0.004
SF RF(IU/ml)	5.26 ± 3.47	27.03± 33.57	0.0097
+ve SF IL-27 (pg/ml)	1 (5.56%)	9 (45%)	0.009
+ve SF IL-33 (pg/ml)	0 (0%)	10 (50%)	0.0005

Comparing serum and SF markers in RA group and OA group, there was no significant difference between serum and SF CRP, RF, IL-27 or IL-33 in both groups.

In RA group, there was a significant positive correlation between serum IL-27 and DAS28 (r = 0.54, p = 0.046) and between serum IL-27 and serum CRP (r = 0.64, p = 0.013). Also, serum IL-33 showed significantly positive correlations with DAS28 (r = 0.68, p = 0.015) and serum RF (r = 0.61, p = 0.036). No other significant correlation was found.

DISCUSSION:

RA is the most common autoimmune inflammatory arthritis, affecting approximately 1% of the population [13].Also,OA is the most common form of arthritis, commonly affects the knee joint, contributing to pain and functional limitations, making knee OA the leading cause of lower extremity disability among older adults (Misra et al., 2013).The pathogenesis of RA is a complex process mediated by an interdependent network of cytokines, prostanoids and proteolytic enzymes. The levels of representative proinflammatory cytokines are increased in patients with RA compared with other forms of arthritis [28].

As the reviews still in debate about the role of IL-33 and IL-27 in RA regarding their proinflammatory and anti-inflammatory effects, so we aimed to investigate the immunopathological roles of IL-27 and IL-33 in RA by estimating their serum and SF levels in RA patients and comparing them to OA and control groups.

IL-27 has both stimulatory effects on Th1 and inhibitory effects on Th1, Th2, and Th17 subsets of T cells as well as the expansion of inducible regulatory T cells, giving its pro-inflammatory and anti-inflammatory properties, thus, it is important to determine whether IL-27 plays a pathogenic or protective role in RA [21]and [51].A high concentration of IL-27 induces the production of IL-6 and inflammatory chemokines from fibroblast-like synoviocytes of RA, providing a novel immunopathological mechanism of IL-27 mediated joint inflammation in RA [52]. In the present study, serum IL-27 was detected in 45.16% (14 of 31)

of RA patients, which was significantly higher than its detection percentage in OA patients (2 of 23, 8.7%) and control groups (2 of 15, 13.33%) (P = 0.0047). Serum IL-27 showed significantly positive correlations with both DAS28 (r = 0.54, p = 0.046) and CRP (r = 0.643, P = 0.013) in RA group, no other significant correlation was found.Also, SF IL-27 was detected in 45 % (9 of 20) of RA patients, which was significantly higher than its detection percentage in OA patients (1 of 18, 5.56%) (P = 0.009) without any other significant correlation.Our results were parallel to that of [52] and [43]who reported elevated serum IL-27 levels in RA patients compared with normal controls suggesting a possible therapeutic significance for IL-27 in RA, and the latest found a significant positive correlation between IL-27 and DAS28 (r = 0.402, P = 0.025) and ESR (r = 0.401, P = 0.026)without correlation withCRP,RFtiteror theother clinical parameters, including, the number of swollen or tender joints.

Regarding the expression level of IL-27, Shahrara et al., [42]observed that IL-27 mRNA levels were also significantly higher in RA SF macrophages compared with control macrophages and concluded that therapy aimed at enhancing IL-27 or IFN-γ may be an attractive approach to suppress TH-17 cell polarization in RA. These results can give a higher spectrum of our search as in our work, we used cheaper and simpler technique and their results go in concordance to our results.

The work of Tanida et al., `[47]agreed with us in that the level of IL-27 was significantly higher in RA SF than OA SF. However, the plasma levels of IL-27 among RA, OA patients and healthy volunteers were equivalent.

On an experimental bases, Baek et al., [10] measured expression of IL-27 protein in the synovial tissue of knee joints in mice after clinical onset of collagen-induced arthritis (CIA). Their results showed that IL-27 expression increased markedly from day 47 after the initial collagen injection. Also, Cao et al., [12] found that IL-27 has led to the development of arthritis by inducing the differentiation of naïve T cells into Th1 cells. Additionally, IL-27Ra$^{-/-}$ mice displayed reduced severity and delayed onset of proteoglycan-induced arthritis, which was associated with decreased interferon expression.

All these findings together with the present study support the proinflammatory role of IL-27 in RA which may be exerted through inducing the differentiation of naïve T cells into Th1 cells.

IL-27 is unique in that it induces Th1 differentiation while simultaneously suppressing immune responses. The immunosuppressive effects of IL-27 depend on inhibition of the development of Th17 cells and induction of IL-10 production. IL-27 exerts potent anti-inflammatory effects in several infectious and experimental autoimmune models [16]. This data supported by an experimental studies by Niedbala et al.,[33], Gabay and Mcinnes, [20] and Pickens et al., [38] whoproved that IL-27 can significantly attenuate the severity of disease using a murine model of CIA or postpone the disease development suggesting protective roles for IL-27 in the pathogenesis of RA.Considering the heterogeneity of the cellular targets and the complexity of the ligand-receptor systems, defining the biology of IL-27 still poses a challenge and dictates the need of further research in order to reconcile that paradox [3].

Even though the immune protective mechanism of IL-27 proved in murineexperimental studies, that effect is not yet proved in human, giving rise of the proinflammatory effect than the antiinflammatory mechanism that go in parallel to our study that gave no correlation between IL-27 and RF that means low humoral immunity and low immunoprotective mechanism,meaning that anti IL-27 can be used as therapeutic target in RA not only a part of the immunopathogenesis of this autoimmune disease.

The IL-1-family-related cytokine, IL-33, was detected at high levels in experimental inflammatory arthritis and in the early phase of human RA, and was reported to exert profound pro-inflammatory effects in several experimental autoimmune models. Moreover, administration

of IL-33 leaded to the development of severe inflammatory arthritis, suggesting that IL-33 may be therapeutically relevant in RA[56].Synovial fibroblasts are believed to be one of the main sources of IL-33 in RA, producing huge amounts of IL-33 in the presence of TNF-α stimulation in vitro. Additionally, in vivo data showed that administration of IL-33 exacerbates experimental arthritis [54].

In the present study, serum IL-33 was detected in 38.71% (12 of 31) of RA patients, which was significantly higher than its detection percentage in OA patients (21 of 23, 4.35%) and control groups (1 of 15, 6.67%) (P = 0.0027). Serum IL-33 showed significant positive correlation with DAS28 (r = 0.678. p = 0.015) and RF (r = 0.609, P = 0.036) in RA group, no other significant correlation was found.Also, SF IL-33 was detected in 50 % (10 of 20) of RA patients, which was significantly higher than its detection percentage in OA patients (0 of 18, 0%) (P = 0.0005) without any significant correlations, so that IL-33 could be used as a prognostic marker. Perhaps this finding raised the possibility that IL-33 may contribute to abnormal B cell autoimmunity.

Our study met the studies of Matsuyama et al., [27],Mu et al., [30], Hong et al., [23],Talabot-Ayer et al., [45],Xiangyang et al., [53] and Tang et al., [46]whofound that serum and SF IL-33 levels were higher in RA than in OA and control groups supporting the idea that IL-33 is implicated in the pathogenesis of RA.In spite of that, the local expression of IL-33 in the synovium was observed at similar variable levels in RA, psoriatic arthritis and OA, suggesting that inflamed joints do not represent the primary source of elevated serum and SF levels of IL-33 in RA [45].

As regard to the IL-33 correlations in RA, our study met the results of a significant correlation with disease activity [27,25] and RF [30,53] while our results were not supported by Tang et al., [46] who found significant correlations regarding disease activity and RF with synovial fluid IL-33.

IL-33 drives production of Th2-associated cytokines including IL-5 and IL-13, which can promote B cell function such as autoantibody production like RF [41].Thus IL-33 may be indirectly involved in B cell-mediated pathology in RA.

Moreover, IL-33 may also contribute to the antibody overproduction by inducing mast cell activation, as it is one of the strongest stimuli for mast cells that reside in the synovial tissue. Both human and animal studies have shown that extracellular IL-33 could stimulate the maturation and activation of mast cells [5].Additionally, Mast cells have been confirmed to be a cellular link between autoantibodies and inflammatory arthritis [34]. This might be the mechanism underlying our data that IL-33 level is associated with RA-related autoantibodies. In support of this hypothesis, IL-33 contributes to the antibody response and the severity of inflammation in a serum-induced arthritis mouse model that is mast cell-dependent [54].So,the present data support the pleiotropic effect of the new cytokine IL-33and give new evidence on the involvement of IL-33 in abnormal humoral immunity (anti-inflammatory effect)with profound proinflammatory effect in spite of positive correlation with RF in many searches including us and may ultimately help in understanding the complex issue of autoimmunity in RA.Thus, neutralization of this cytokine may help as new therapeutic approach for RA.

The results of the present study showed differences in the laboratory findings of WBCs, ESR, hsCRP, RF, IL-27 and IL-33 in RA (inflammatory arthritis) compared with OA (degenerative arthritis). The mean WBCs was significantly higher in RA patients than OA patients and control group. Leukocytosis (WBCs >10 K/µl) was found in 25.8% (8 of 31) of RA patients. Our finding was in agreement with Richie and Francis, [39], Rindfleisch and Muller [40] and Yazici et al., [55] who stated that WBCs might increase in RA. This may be explained by that RA is an inflammatory disease and the elevated WBCs typically reflect the

normal response of bone marrow to inflammatory process or steroid therapy [1].

The acute-phase reactants, CRP and ESR, are used as a surrogate marker of inflammation, easy to perform, routinely available and the most widely used biological markers for assessing disease activity in RA [32].

In the current work, the mean serum CRP level and ESR were significantly higher in RA patients than OA patients and control group and the mean SF CRP was significantly higher in RA than OA patients. Our results were supported by Bramlage et al., [11],Morovic-Vergles et al., `[29] and Zamani et al., [57]who found that serum CRP level and ESR were significantly higher in RA than OA.

RF is a very old serological marker for diagnosis of RA, taken as a non-specific marker of RA [44]. In our study, the mean serum level of RF was significantly higher in RA than OA patients and control group and the mean of SF RF was significantly higher in RA than OA patients. These results were in accordance with Khalifa and Abdelfattah, [26] and Hui et al., [24], whose results indicated that RF was significantly elevated in RA patients than non- RA patients. Also, caspi et al, [14] found that RF was significantly increased in the serum and synovial fluid of patients with RA in comparison with another inflammatory joint effusion condition (Psoriatic Arthritis) and a noninflammatory synovial fluid condition (OA).

CONCLUSION:

The significantly higher detection levels of both IL-33 and IL-27 in RA patients than OA and control groups; support their proinflammatory role in this autoimmune disease as a part of its complicated autoimmune abnormality. In RA group, both serum IL-27 and IL-33 correlated with disease activity, reflecting their association with poor prognosis of the disease, while the former also correlated with the inflammatory marker CRP and the later also correlated with the autoantibody RF, giving new evidence on the involvement of IL-33 in abnormal humoral immunity hence anti-inflammatory property.

Competing interests: The authors declare that they have no competing interests.

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