EFFECT OF MENOPAUSE ON DIFFERENT CYTOKINES LEVELS AND PROPORTION OF T-REGS IN RHEUMATOID ARTHRITIS (RA) IRAQI WOMEN

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Abstract

The aim of the study to determination of the precise percentage of T-regs cells in rheumatoid arthritis (RA) patients to discover the manipulating role of menopause statues (pre and post) in the percentage of these cells in patients and may eventually influence the development of rheumatoid arthritis. Also to explore the possibility of using some cytokines as a marker for disease activity by evaluating the direct potential role of IL-35 and the indirect potential contribution of IL-33 and its soluble receptor sST2 on the percentage of effectors T-regs cells.

This study was conducted on a total of 90 women: 60 of them were RA women patients (30 premenopausal and 30 postmenopausal women) and 30 healthy controls (15 premenopausal and 15 postmenopausal women). All RA patients and controls were diagnosed by measuring (ESR, CRP, Anti CCP and RF). IL 33, sST2 and IL-35 level was measured in the serum of both RA patient group and control group by ELISA, were the T-regs percentages evaluated in whole blood by flowcytometery. The results showed that the total mean concentration of IL-33/sST2 in women infected with RA was significantly ($p<0.01$) increased in comparison to total control group. However, postmenopausal women scored highly significant ($p<0.01$) increase of IL-33/sST2 in comparison to premenopausal women both stages declared a significant ($p<0.01$) increase in comparison to control group. The total mean concentration of IL-35 and T-regs percentages in women infected with RA was significantly ($p<0.01$) decreased in comparison to total control group. However, postmenopausal women scored highly significant ($p<0.01$) decreased of IL-35 and T-regs percentages in comparison to premenopausal women. Serum IL 33/sST2 showed significantly positive correlations with DAS 28, while IL-35 and T-regs showed significantly negative correlations with DAS 28. It can be conclude that IL 33/sST2 has an important pro-inflammatory role in the pathogenesis of RA, while IL-35 and T-regs has an important anti-inflammatory role correlation with disease activity. Highly positive significant linear correlation was seen between T-regs and IL-35 they may become potential therapeutic targets for RA.

Key words: DAS 28, Interleukin 33, IL-35, T-regs, Flowcytometery, Rheumatoid arthritis.

Introduction

Immunopathology of RA is characterized by a complex interplay between adaptive and innate immune system, along with responses mediated by synovial resident cells. Indeed, the demarcation between these immunological compartments is a simplistic and artificial schematization as in real life they crosstalk and integrate to form an inextricable network (Firestein and McInnes, 2017). Rheumatoid arthritis is usually more common in women than in men and is considered to be the fourth principal cause of women's illness (Mollard *et al*., 2018). Menopausal age as predictor of disease severity, more studies need to be conducted to be able to draw any conclusions that may be used as a basis for disease management. Sex hormones are the major factors affecting the differences between the female and male immune systems, Due to the incidence of hormone receptors on immune cells (Hughes and Choubey, 2014).

In RA, the persistent and chronic inflammation suggests that there is a failure in the mechanisms that control immune responses. The failure of T-regs to modulate inflammation in RA has been attributed to either the reduced responsiveness of immunity to suppression or defective function of T-regs themselves (Monte *et al*., 2008; Horwitz *et al*., 2019). Some studies have

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reported that IL-35 can carry out significantly in the progress of RA. Accordingly, the strong relationship of this cytokine with inflammatory autoimmune disorders at multiple levels encourages researchers to study IL-35 in relation to these diseases, taking into account their regulatory capacity in these disorders (Dambuza, 2017). Some studies have shown an increase in T-reg in the presence of IL-35 (Su *et al.*, 2018).

Some studies show that cells do not release IL-33 unless they get injured or became damaged (Nile *et al.*, 2010). Furthermore, *in vivo* they found that IL-33 was able to induce the expression of many cytokines such as IL-4, IL-5 and IL-13 and to promote severe pathological alterations in mucosal tissue (Cai, 2020). There are at least two splice variants of ST2 translated in humans, sST2 (soluble form) and ST2L (long trans-membrane form) IL-33 and its receptor ST2 (Le, 2000; Odegaard *et al.* 2016). sST2 can also bind IL-33 as a 'decoy' receptor which prevents IL-33 and ST2L from being combined and thus blocks the functional effect of IL-33 (Hayakawa *et al.*, 2007).

Martials and Methods

Samples collection of this study was started in October 2018 to January 2019. The objective of this study was focus on two separated categories of women with rheumatoid arthritis: the premenopausal RA cohort and the postmenopausal RA. Seven milliliters of blood Samples of healthy control women and RA patients who attended the rheumatology clinic were collected at Baghdad University Hospital in Baghdad Medical City Hospital (Baghdad, Iraq) and met the ACR / EULAR criteria for RA. Each collected sample was dispensed in two tubes: EDTA tubes for whole blood parameters assays and Gel tubes for serology measurements. Seventy Newly diagnosed RA patients were used (within two year of the onset of the first symptoms). At the same time, 30 health controls (HC) were selected from the Iraqi population register randomly in order to match age, gender, economic and residential status. All samples were taken in the context of a case study group (RA) for all laboratory investigations.

The kits were used to test the level of IL-33 (provided by ABCAM, UK: Cat No. ab223865), Human Soluble Stromelysin-2(sst2) provided by MyBiosource, USA (Cat No: 773449), IL-35 provided by MyBiosource, USA: Cat.No: 251198796T) based on the principle of biotin double antibody sandwich technology enzyme linked Immunosorbent assay (ELISA).

Flowcytometry is a technique that allows for the semi quantitative measurements on a single cell basis. This

technique in the current study was operated at the Center for Hematology in Al-Yarmouk Teaching Hospital with the assistance of the expert supervising the device and the kits were prepared from (BD Biosciences, USA). The analysis performed by using a basic protocol according to the company's instructions for identifying T-regs cells, Use a minimal cocktail of CD4, CD25 and FoxP3 antibodies.

The antibodies used in flowcytometery procedure of T-regs detection are surface staining of Treg cells with CD4 and CD25 (Cat No: 560249) and Anti-FoxP3 staining with Intracellular method (Cat. No. 560131). Lymphocytes determined by FSC/SSC gating strategy and dead cells excluded from the analysis by using the live/dead cell marker (PI). Lymphocytes analyzed further for their expression of CD25 (x-axis) and CD4 (y-axis) to determine the purity of $CD4^+CD25^+$ T-regs cells and Lymphocytes analyzed further for their expression of CD4 (y-axis) and FoxP3 (x-axis) to determined initial frequency measurement of FoxP3-expressing $CD4^+$ cells among lymphocytes by FlowJo software from BD-Bioscience.

Statistical analysis

The software SAS, (2012) was used to detect the effect of study parameter differential factors. The LSD test (Variation Analysis-ANOVA) was used to compare significantly between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) and correlation coefficient were estimated between variables in this study.

Results

Out of 225 examined women infected with rheumatoid arthritis (RA), only 70 patients were included in this study. This selection was depend on the exclusion of each characteristic that may lead to dispersal the results, such as interference with other chronic diseases,
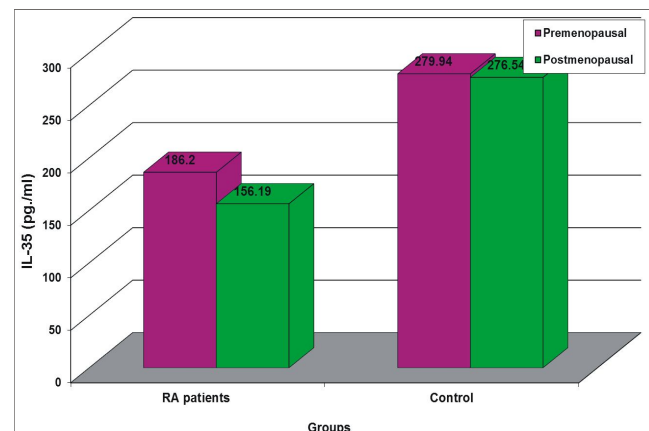

Fig. 1: Comparison between different groups according IL-35 concentrations in women with different menopausal stages.

Table 1: Comparison between Level of IL-35 (Mean± SE) in the studied groups and correlation with Das 28.

| Groups		Mean ± SE	
		IL-35 (pg./ml)	Das 28
Total patients		171.19 ± 1.68	4.14 ± 0.16 ab
Total control		278.24± 6.34	1.54 ± 1.13 c
RA patients	Premenopausal	186.20 ± 0.44 b	3.66 ± 0.09 b
	Postmenopausal	156.19 ± 1.24 c	4.63 ± 0.10 a
Control	Premenopausal	279.94 ± 2.26 a	1.42 ± 0.06 c
	Postmenopausal	276.54 ± 4.12 a	1.66 ± 0.07 c
LSD value		7.017 **	0.246 **
Means with dissimilar letters in similar column differed significantly. ** (P≤0.01).			
Correlation coefficient between Das28 and IL-35			
Variables	Correlation coefficient-r with Das28		Level of Sig.
IL-35	-0.98		**
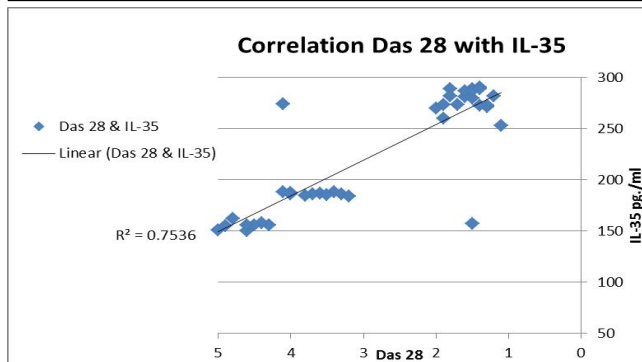
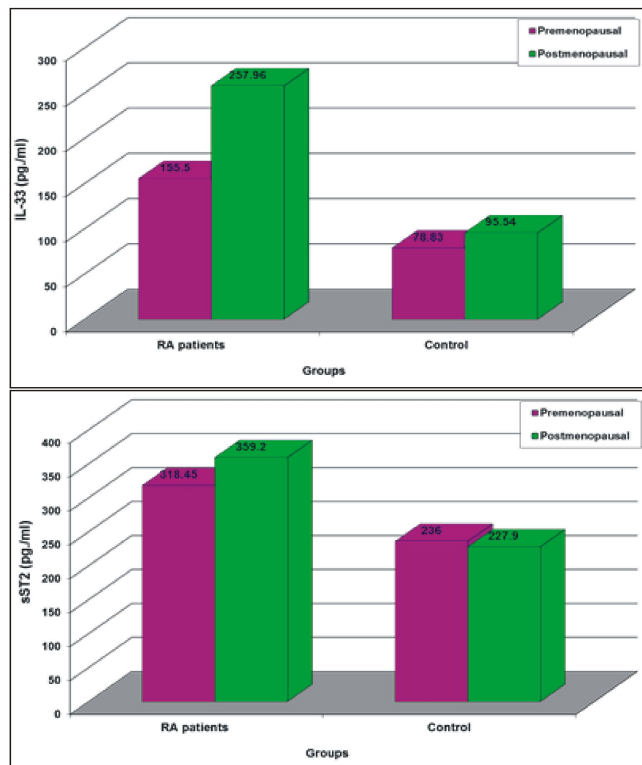**Fig. 2:** Correlations between IL-35 and Das28 score in RA women patients.

Fig. 3: Comparison between different means according IL-33 and sST2 conc. in different menopausal stages.

duration of the illness, smoking, patient without treatment since months ago and each one who had negative RF or CRP or ACCP were excluded. However, this study included thirty women chosen as control with the same age, geographical area and living conditions.

The results showed that the total mean concentration of IL-35 (171.19 ± 1.68 pg. /ml) in women infected with RA was significantly (p<0.01) decreased in comparison to total control group (278.24±6.34 pg./ml). However, postmenopausal women scored highly significant (p<0.01) decrease of IL-35 (156.19 ± 1.24 pg. /ml) in comparison to premenopausal women (186.20 ± 0.44 pg. /ml) and both stages declared a significant (p<0.01) decrease in comparison to control group table 1, fig. 1. According to the disease severity the results showed that highly negative correlation between IL-35 and Das28 score as showed in table 1, fig. 2.

The results showed that the total mean concentration of IL-33 (206.73± 9.61 pg./ml) in women infected with RA was significantly (p<0.01) increased in comparison to total control group (87.185± 3.78 pg./ml). However, postmenopausal women stored highly significant (p<0.01) increase of IL-33 (257.96 ± 7.72 pg./ml) in comparison to premenopausal women (155.50 ± 1.89 pg./ml) and both stages declared a significant (p<0.01) increase in comparison to control groups.

Also, The results showed that the total mean concentration of sST2 (338.82± 12.05 pg./ml) in women infected with RA was significantly (p<0.01) increase in comparison to total control group (231.95± 12.23 pg./ml). However, postmenopausal women stored highly significant (p<0.01) increase of sST2 (359.20 ± 8.57 pg./
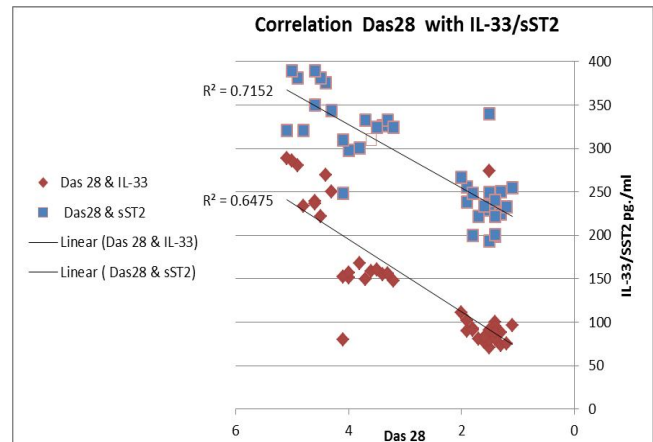

Fig. 4: Correlation between Das28 and IL-33 and its receptor sST2 in RA women patients.

Table 2: Comparison between the level of IL-33 and its receptor sST2 (Mean± SE) in the studied groups and their correlation with Das 28.

Groups		Mean ± SE	
		IL-33 (pg./ml)	sST2 (pg./ml)
Total patients		206.73±9.61 ab	338.82± 12.05 ab
Total control		87.185± 3.78 cd	231.95± 12.23 c
RA patients	Premenopausal	155.50 ± 1.89 b	318.45 ±4.05 b
	Postmenopausal	257.96 ± 7.72 a	359.20 ±8.57 a
Control	Premenopausal	78.83 ± 1.59 d	236.00 ±3.23 c
	Postmenopausal	95.54 ± 2.18 c	227.90 ±8.91 c
LSD value		12.052 **	19.23 **
Mean with different letters in same column differed significantly. ** ($P \leq 0.01$).			
Correlation coefficient between Das28 and other variables			
IL-33		0.93	**
sST2		0.93	**

Table 3: T-regs cells percentage among CD4^{+} T- Cell in PMNCs.

Groups		Mean ± SE of T-regs %
Total patients		1.17±0.2 cd
Total control		4.87±0.10 b
RA patients	Premenopausal	1.34 ±0.08 c
	Postmenopausal	1.00 ±0.12 d
Control	Premenopausal	4.58 ±0.10 b
	Postmenopausal	5.17 ±0.15 a
LSD value		0.338 **
Means with different letters in same column differed significantly. ** ($P \leq 0.01$).		
Correlation coefficient between Das28 and T-regs proportion		
T-regs		-0.92

ml) in comparison to premenopausal women (318.45 ± 4.05 pg./ml) and both stages declared a significant (p<0.01) increase in comparison to control groups. According to the disease severity, the results showed that there was a highly significant linear positive correlation between IL-33 and its receptor (sST2) with Das28 score as showed in table 2, fig. 3 and 4.
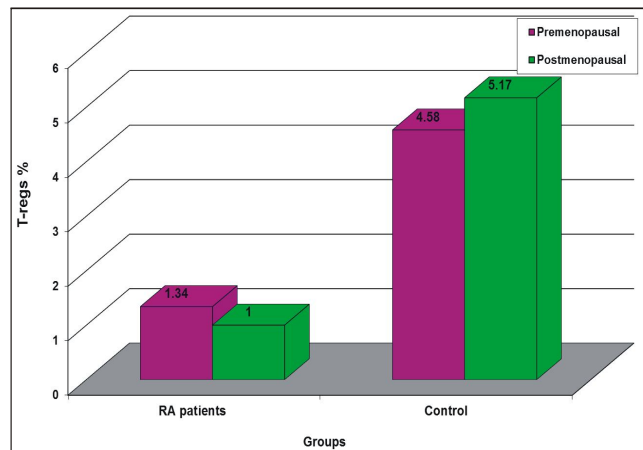

Fig. 5: Comparison between different means according T-regs proportion in different menopausal stages.

The results showed that the total mean percentages of T-regs (1.17± 0.2 %) in women infected with RA was significantly (p<0.01) decreased in comparison to total control group (4.87± 0.10 %). However, postmenopausal women stored highly significant (p<0.01) decrease of T-regs percentages (1.00 ± 0.12%) in comparison to premenopausal women (1.34 ± 0.08%) and both stages declared a significant (p<0.01) decrease in comparison to control group table 3, fig. 5. According to the disease severity the results showed that highly negative correlation between T-regs and Das28 score as showed in table 3, fig. 6. Moreover, the results showed that highly significant positive correlation between T-regs and IL-35 and both of them sharing the same regulatory role in RA disease, fig. 6.
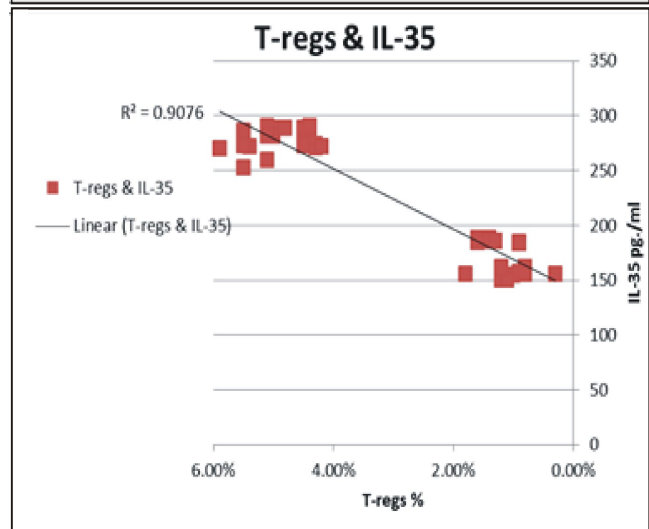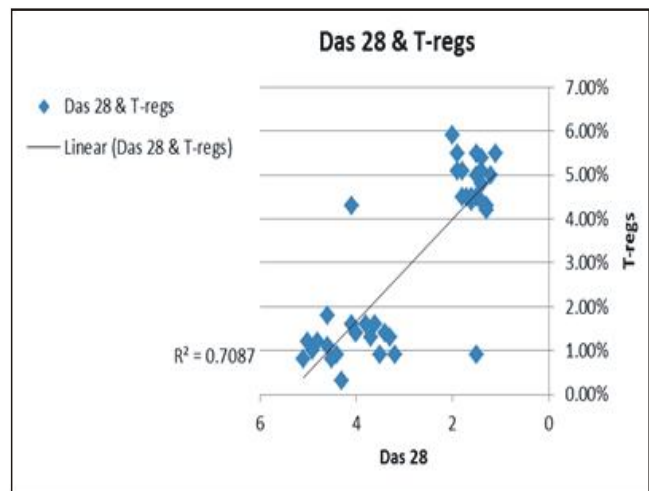

Fig. 6: Correlation of Das 28 with T-regs proportions and IL-35 levels in RA women patients.
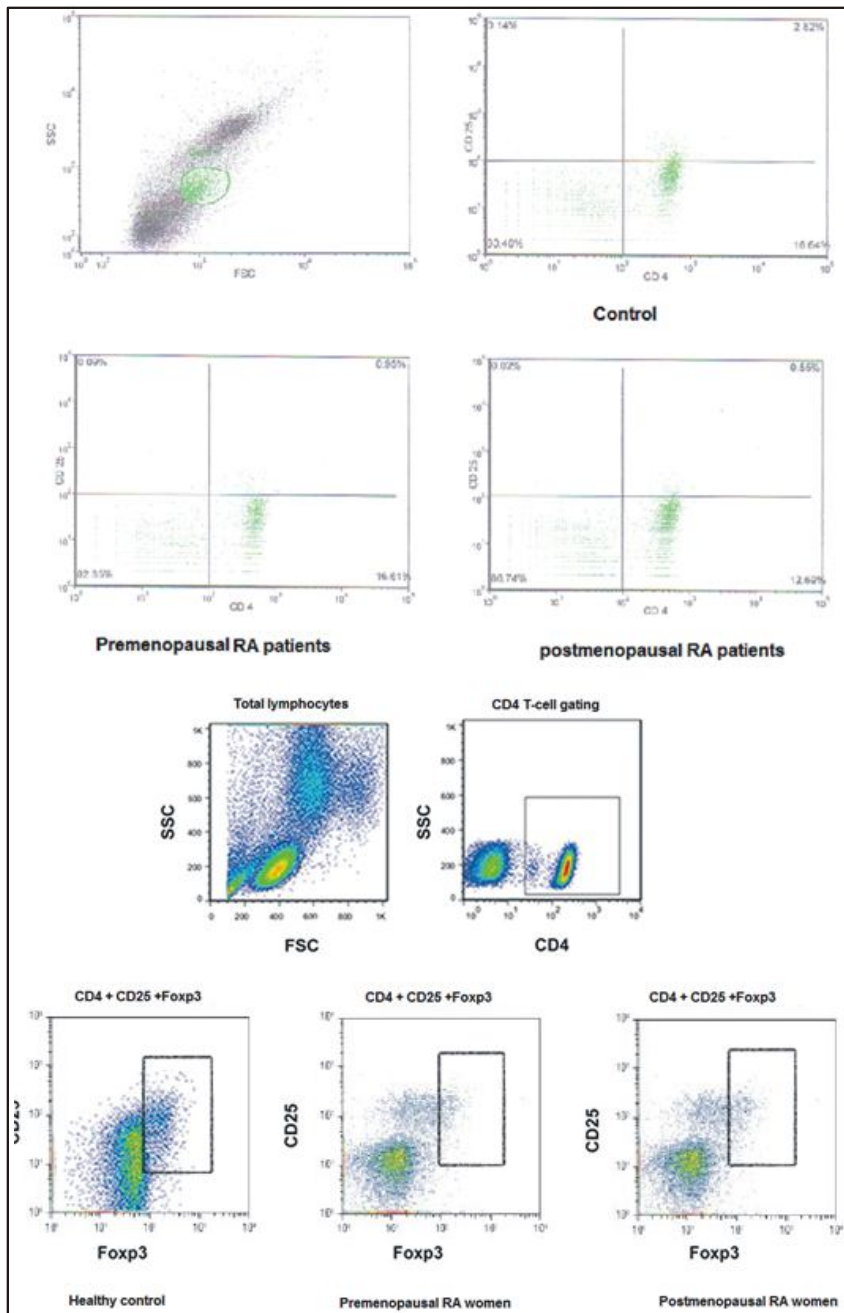
Fig. 7: Flowcytometery analysis.

Discussion

These presented results concluded that IL-35, an important anti-inflammatory cytokine, may contribute in the regulation of the pathogenesis of Rheumatoid Arthritis, especially with disease activity and confirm the regulatory role of interleukin-35 in autoimmune diseases including rheumatoid arthritis. These results agreed with previous results who mentioned that high serum IL-35 levels were significantly associated with low disease activity and their multivariate and univariate analysis revealed that the high serum IL-35 concentrations were associated with low Das28 and ESR (Li *et al*., 2019). Depending on very

homogeneous samples of recruited RA women patients, the results found that serum IL-35 levels were significantly lower in RA patients women especially in those with postmenopausal stage and this findings agreed with previous results concluded that low serum IL-35 levels are accompanying with bone damage, low BMD, high bone metabolism and lower levels of vitamin D3 in postmenopausal women with RA and this proposing that IL-35 may represent a novel therapeutic target RA with bone loss (Li *et al*., 2019).

Previous study stated that the low concentrations of IL-35 in RA patients, when compared with healthy control, specifying that the IL-35 expression can be inhibited in patients with RA. It was also discovered that IL-35 concentration is negatively correlated with RF, neutrophil proportion and bone degradation in RA patients. These findings proposed a likely protective function for IL-35 in the pathogenesis of RA, especially in the progression of inflammation and the studies must concentrate on the protective effect mechanism of IL-35 in the development of RA or explore the therapeutic significance of IL-35. Study done by Tanta University Hospitals concluded that IL-35 was diminished with certain illness markers such as increased RF, ESR and CRP representing its role in the inflammatory developments accompanying with RA disease progress (Ning *et al*., 2015). From all previous discussed studies it is believed that IL-35 can help reduce the severity of the RA disease.

The current results agreed with previous study done by Farag *et al*., (2017) who found that serum IL-33 levels in RA patients were significantly higher than that in osteoarthritis patients. This might idea to differentiate the disease mechanism involved in these arthropathy illness, proposed that induction of IL-33 expression by fibroblasts might be commonly observed in damaged or inflamed tissues (Akl *et al*., 2019). Another agreed study found that IL-33 was raised in patients with RA and was associated with high Das28, RF, anti CCP antibodies, CRP and ESR. However, they found significant correlation

between IL-33 and disease activity (Salama *et al.*, 2017).

The present findings found the postmenopausal RA women have higher significant IL-33 concentrations in comparison with premenopausal RA women and this fact agree with previous study concluded that postmenopausal women have higher levels of the pro-inflammatory cytokines for example (TNF-α, IL-1β and IL-8) and lower serum levels of anti-inflammatory cytokines such as (IL-20) in comparison with premenopausal women (Malutan *et al.*, 2014).

Duan *et al.,* (2013a) concluded that IL-33 plays an important role in the progress and development of autoimmune diseases and IL-33 expression was changed in the blood of active patients, or this could be related to inflammatory cytokines such as IL-1β and TNF-α. Some studies report that cells do not release IL-33 unless they become injured or undergo necrosis (Cayrol and Girard, 2018).

Okragly *et al.,* (2016) demonstrated that in the normal physiological circumstances the absence of IL-33 has no negative effect on bone homeostasis, whereas high cytokine levels IL-33 cause rapid and severe bone destruction, this is a direct effect of IL-33 on bone reabsorption cells, or an indirect effect attributable to other IL-33-stimulated pro-inflammatory cytokines, for example IFN-C and IL-6 and this interctions of the cytokine network reveals IL-33's effects on bone physiology (Cayrol and Girard, 2016). Up to now, effort on IL-33 and autoimmune rheumatic diseases primarily focuses on the level of expression of IL-33 and disease severity, but the underlying mechanism and related clinical therapy are still under investigation. Depending on the above-mentioned results, this study may conclude the clinical application of IL-33 / ST2 related therapy in patients receiving treatment (Cai, 2020). The role of IL-33 in severity of RA disease declared by several studies has revealed expanded IL-33/ST2 generation in the serum and synovial fluid of patients with RA disease. The level of articulation of IL-33 and ST2 appears to correspond to the movement of RA sickness. IL-33 design encourages the generation of expert cytokines (IL-1β, MCP-1 and IL-6) and fuels collagen-induced arthritis (CIA) progression in mice, this information show that revocation of IL-33/ST2 opened up incendiary reaction connects to a curative focus for RA disease (Veeraveedu, 2017).

The synovial fluid is important sites to irritation in RA; from which cytokines are enter into the fundamental course. These cytokines are in a state to adjust the working of skeletal muscle, fatty tissue, liver and vascular endothelium and produce a range of pro-atherogenic changes that includes insulin opposition, a trademark dyslipidemia, oxidative pressure and endothelial brokenness (Nguyen, 2017).

It has been demonstrated that the receptor of IL-33 assumes to be a critical part in immune system disease, Aggravation and contamination. IL-33 has recently been shown to change the manifestations of RA joint pain, SLE and other diseases of the immune system. They can inspire gainful or hindering impacts that rely on the setting of an ailment. Ponders on IL-33may give another thought and focus to the treatment of diseases in the immune system (Lax, 2015).

These conclusions of previous studies stated that IL-33 had similarity with the 'alarmin' family of proteins which are also only released in response to cell damage that is released after cells or tissue necrosis to warn the immune system against damage to tissues. This family includes High mobility group box 1 protein (HMGB1), defensins, heat shock proteins and IL-1α (Cayrol and J.P. Girard, 2009; Holgado, 2019). However, in spite of being characterized as an (alarmin), studies have found that under certain situations IL-33 is released by viable cells such as monocytes, macrophages, fibroblasts and glial cells (Talabot *et al.*, 2009; Mishra, 2019). Depending on the current considerate, IL-33 has similarity to IL-1β and HMGB1 which also have been shown to have transcriptional regulatory properties (Lotze and K.J. Tracey, 2005). Therefore, like IL-1α and HMGB1, IL-33 is a double function protein acting as a pro-inflammatory extracellular cytokine and as nuclear factor with intracellular transcriptional regulatory properties (Gautier, 2016). Also, in therapeutic approaches the use of an antibody against IL-33 may have non-specific effects which could reduce RA disease severity. The totality of data, however, suggests that the IL-33 / ST2 axis is involved in Th2-mediated immune responses in RA patient joints (Murdaca, 2019).

Komai *et al.,* (2016) Suggested that IL-33 may stimulate Th-1 cell differentiation by following mechanisms; (1) IL-33 signals promoting IL-12 and ST2-based Th1 differentiation; This is demonstrated by the observation that IL-33 alone cannot polarize Th1 cells (2) whether IL-33 and IL-12 synergize with Th1 cell polarization is unknown. This suggests that IL-33 and IL-12 could do so by improving both the expression of ST2 and IL-12R in initially activated CD4^{+} cells. Also, they found that the enhanced ST2 expression induced by IL-33 and IL-12 was down-regulated 72 h after Th1 polarization *in vitro*. Furthermore, IL-33 may also act as a novel adjuvant in vaccination against infectious diseases and cancer.

The present results showed that significant increases of sST2 in serum of RA women patients especially in postmenopausal RA women, besides that, it was strongly correlated with Das28 and associated inflammatory sings. This results agree with previous study suggested that both soluble ST2 considered as a marker of RA severity and patient disability and had negative association with metabolic syndrome is in agreement with the possible protective role of this cytokine receptor (Pinto and Andrade, 2017).

Soluble-ST2 is one of many parameters linked to inflammation that are changed in RA and these results agree with previous results that explain the statement of sST2 is matched with the continuous fibrosis and inflammation phase Mineralocorticoid receptor enemies have been shown to lessen cardiovascular fibrosis by balancing IL-33 / ST2 axis and flagging galectin-3. These findings show that IL-33 / ST2 takes on a defensive role in cardiovascular fibrosis and sST2 adversely guides this pathway as an IL-33 bait receptor (Hong, 2011). sST2, expressed in mammary tumors, embryonic cells and fibroblasts, was like to the extracellular region of ST2L and competes for IL-33 binding, inhibiting receptor signaling (Hayakawa et al., 2007). A previous study has demonstrated that the levels of sST2 were correlated with disease activity in patients with other autoimmune diseases such as SLE and inflammatory bowel disease (Italiani et al., 2018). These Cytokines receptors regulate important biological progressions such as hematopoiesis or immune response and are involved in the pathogenesis of many illnesses (Italiani et al., 2019). Therefore, any rise in their concentrations suggests stimulation of pathways involved in an infiammatory response or disease progress. That is why cytokines may serve as potential biomarkers of various autoimmune diseases and changes of their levels may be used in follow-up assessment. Similarly, the study done on the juvenile patients with idiopathic arthritis, found that the levels of soluble ST2 were higher than those in healthy controls. In this case, levels of sST2 correlated well with the severity of disease and reduced during the phase of remission (Ishikawa et al., 2013). In this study, sST2 is related with these findings, by which we can presume sST2 can be recognized as a disease biomarker. However, whether the increased sST2 might serve as a bio-marker of RA or as a regulative mechanism that contributed to the disease pathogenesis needs to be further investigation.

The role of IL-33 on RA can be inverted by sST2 which act as decoy receptors (Decoy receptor means protein that can bind functional cellular receptor ligands, effectively decreasing the ligand concentration presented to the active receptor). Why did sST2 increase in RA especially in the postmenopausal patients? A regulatory role attributed to sST2 could be a possible answer, suggesting that this protein might be playing a negative feedback mechanism to control inflammation. In addition, current involvement and peripheral arthritis are not only the identification of RA but also can reflect disease severity.

The present study found that IL-35 had positive correlation with T-regs proportions this agree with previous results that concluded IL-35 is anti-inflammatory cytokine suppressing the immune system by the expansion of T-regs and prevent of Th-17 cell expansion (Ntolkeras, 2019).

The present study were in line with meta- analysis of previous studies done by Morita et al., (2016) who showed that the percentage of T-regs defined for $CD25^+$ $CD4^+$ in RA patients was lower than that of control subjects (Morita et al., 2016). Kailashiya et al., (2019) concluded that flowcytometry results identified difference in percentages of T-regs in blood samples of RA and SLE groups. These differences in levels and associations with various serum markers and symptoms (showing significant association only in SLE but not in RA) also suggest that T-regs play different role in pathogenesis and clinical performances in different autoimmunity diseases.

Previous study proposed that in an inflammatory disorder, it was relatively possible that T-regs in the presence of the different pro-inflammatory cytokines will become unbalanced and convert to pathogenic T-cells. Serum cytokine environment of active RA illness is not in favor of the development of Tregs cells (Chavele and Ehrenstein, 2011). T-regs which are specialized in the maintenance of immune tolerance and homeostasis and which secrete several immune-suppressive and anti-inflammatory cytokines such as IL-10, IL-27, Transforming growth factor-β (TGF-β) and IL-3. Deficiencies in T-regs were suggested to lead to the immunological abnormalities seen in most autoimmune diseases (Arenas et al., 2015). T-regs functional deficiency was considered to be the origin of autoimmune diseases such as RA. Nevertheless, the peripheral immune tolerance role of T-regs in patients with RA was not adequately explained in previous research and the proportion of RA-patients with T-regs in PB to clarify T-regs status in RA. The current study performed detection of the percentage of T-regs among $CD4^+$ T cells in RA-patients by using most accurate markers includes ($CD4^+CD25^+FOXP3^+$) by flowcytometery.

Conclusion

Cytokine estimations considered as potential biomarkers of RA autoimmune disease and their levels may be used as a follow-up assessment. Highly negative significant linear correlation was seen between IL-35 and Das28. while, highly positive significant linear correlation was seen between IL-33 and its soluble receptor (sST2) with disease severity (Das28) at menopause duration. Postmenopausal RA women scored highly significant decrease of T-regs cells in comparison to premenopausal one and this correlated by a linear significant correlation with disease activity (Das28). Highly positive significant linear correlation was seen between T-regs and IL-35. also, highly positive significant linear correlation was seen between T-regs percentage and sex hormones levels. Flowcytometry is the most important analysis for T-regs cells in PBLs.

References

Akl, N.E.E., S.M. Abd El-Halim, M.M. Mabrouk, D.S. Ashkar and S.A. Hablas (2019). "Role of interleukin-35 in rheumatoid arthritis pathogenesis and its relation to disease activity and joint damage," *Egypt Rheumatol. Rehabil.*, **46(3):** 177.

Arenas-Ramirez, N., J. Woytschak and O. Boyman (2015). "Interleukin-2: biology, design and application," *Trends Immunol.*, 36(12): 763-777.

Cayrol, C. and J. Girard (2018). "Interleukin 33 (IL 33): A nuclear cytokine from the IL-1 family," *Immunol. Rev.*, **281(1):** 154-168.

Chavele, K.M. and M.R. Ehrenstein (2011). "Regulatory T-cells in systemic lupus erythematosus and rheumatoid arthritis," *FEBS Lett.*, **585(23):** 3603-3610.

Cai, H. (2020). "Salidroside suppresses group 2 innate lymphoid cell-mediated allergic airway inflammation by targeting IL-33/ST2 axis," *Int. Immunopharmacol.*, **81:** 106243.

Cayrol, C. and J.P. Girard (2009). "The IL-1-like cytokine IL-33 is inactivated after maturation by caspase-1," *Proc. Natl. Acad. Sci.*, **106(22):** 9021-9026.

Duan, L., J. Chen, F. Gong and G. Shi (2013). "The role of IL-33 in rheumatic diseases," *Clin. Dev. Immunol.*, **2013:** 5-9, doi: 10.1155/2013/924363.

Dambuza, I.M. (2017). "IL-12p35 induces expansion of IL-10 and IL-35-expressing regulatory B cells and ameliorates autoimmune disease," *Nat. Commun.*, **8(1):** 1-12.

Farag, A.M.A.E.A., N.M. El-Gazzar, M.A.A. El-Hawa and M.A.S. Attia (2017). "Study of interleukin 33 in rheumatoid arthritis versus osteoarthritis patients," *Egypt. Rheumatol. Rehabil.*, **44(4):** 159.

Firestein, G.S. and I.B. McInnes (2017). "Immunopathogenesis of Rheumatoid Arthritis," *Immunity*, **46(2):** 183-196, doi: 10.1016/j.immuni.2017.02.006.

Gautier, V. (2016). "Extracellular IL-33 cytokine, but not endogenous nuclear IL-33, regulates protein expression in endothelial cells," *Sci. Rep.*, **6:** 34255.

Hughes, G.C. and D. Choubey (2014). "Modulation of autoimmune rheumatic diseases by oestrogen and progesterone," *Nat. Rev. Rheumatol.*, **10(12):** 740-751, doi: 10.1038/nrrheum.2014.144.

Horwitz, D.A., T.M. Fahmy, C.A. Piccirillo and A. La Cava (2019). "Rebalancing Immune Homeostasis to Treat Autoimmune Diseases," *Trends Immunol.*

Hayakawa, H., M. Hayakawa, A. Kume and S. Tominaga (2007). "Soluble ST2 blocks interleukin-33 signaling in allergic airway inflammation," *J. Biol. Chem.*, **282(36):** 26369-26380.

Holgado, A. (2019). "IL-33trap is a novel IL-33–neutralizing biologic that inhibits allergic airway inflammation," *J. Allergy Clin. Immunol.*, **144(1):** 204-215.

Hong, Y.S. (2011). "Measurement of interleukin-33 (IL-33) and IL-33 receptors (sST2 and ST2L) in patients with rheumatoid arthritis," *J. Korean Med. Sci.*, **26(9):** 1132-1139.

Italiani, P. (2018). "IL-1 family cytokines and soluble receptors in systemic lupus erythematosus," *Arthritis Res. Ther.*, **20(1):** 27.

Ishikawa, S., M. Shimizu, K. Ueno, N. Sugimoto and A. Yachie (2013). "Soluble ST2 as a marker of disease activity in systemic juvenile idiopathic arthritis," *Cytokine*, **62(2):** 272-277.

Komai-Koma, M., E. Wang, M. Kurowska-Stolarska, D. Li, C. McSharry and D. Xu (2016). "Interleukin-33 promoting Th1 lymphocyte differentiation dependents on IL-12," *Immunobiology*, **221(3):** 412-417.

Kailashiya, V., U. Singh, R. Rana, N.K. Singh, D. Dash and J. Kailashiya (2019). "Regulatory T cells and their association with serum markers and symptoms in systemic lupus erythematosus and rheumatoid arthritis," *Immunol. Invest.*, **48(1):** 64-78.

Li, Y., L. Yao, S. Liu, L. Xia, H. Shen and J. Lu (2019). "Correlation between Serum IL-35 Levels and Bone Loss in Postmenopausal Women with Rheumatoid Arthritis," *Mediators Inflamm.*, **2019**.

Lotze, M.T. and K.J. Tracey (2005). "High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal," *Nat. Rev. Immunol.*, **5(4):** 331-342.

Li, H. (2000). "The cloning and nucleotide sequence of human ST2L cDNA," *Genomics*, 67(3): 284-290.

Lax, A. (2015). "Mineralocorticoid receptor antagonists modulate galectin-3 and interleukin-33/ST2 signaling in left ventricular systolic dysfunction after acute myocardial infarction," *J.A.C.C. Hear. Fail.*, **3(1):** 50-58.

Mollard, E., S. Pedro, E. Chakravarty, M. Clowse, R. Schumacher and K. Michaud (2018). "The impact of menopause on functional status in women with rheumatoid arthritis," *Rheumatology* (Oxford)., **57(5):** 798-802, doi: 10.1093/rheumatology/kex526.

Monte, K., C. Wilson and F.F. Shih (2008). "Increased number and function of FoxP3 regulatory T cells during experimental arthritis," *Arthritis Rheum. Off. J. Am. Coll. Rheumatol.*, **58(12):** 3730-3741.

Malutan, A.M., M. Dan, C. Nicolae and M. Carmen (2014). "Proinflammatory and anti-inflammatory cytokine changes related to menopause," *Prz. menopauzalny= Menopause Rev.*, **13(3):** 162.

Mantovani, A., C.A. Dinarello, M. Molgora and C. Garlanda (2019). "Interleukin-1 and related cytokines in the regulation of inflammation and immunity," *Immunity*, **50(4):** 778-795.

Murdaca, G. (2019). "IL-33/IL-31 Axis in Immune-Mediated and Allergic Diseases," *Int. J. Mol. Sci.*, **20(23):** 5856.

Morita, T., Y. Shima, J.B. Wing, S. Sakaguchi, A. Ogata and A. Kumanogoh (2016). "The proportion of regulatory T cells in patients with rheumatoid arthritis: a meta-analysis," *PLoS One*, **11(9):** 2016.

Mishra, P.K. (2019). "Sterile particle-induced inflammation is mediated by macrophages releasing IL-33 through a Bruton's tyrosine kinase-dependent pathway," *Nat. Mater.*, **18(3):** 289-297.

Ntolkeras, G. (2019). "On the immunoregulatory role of statins in multiple sclerosis: the effects on Th17 cells," *Immunol. Res.*, 1-15.

Nile, C.J., E. Barksby, P. Jitprasertwong, P.M. Preshaw and J.J. Taylor (2010). "Expression and regulation of interleukin 33 in human monocytes," *Immunology*, **130(2):** 172-180.

Nguyen, P.N. (2017). "Characterization of vascular function of isolated blood vessels in response to high glucose and internal mammary artery from diabetic patients."

Ning, X., Z. Jian and W. Wang (2015). "Low serum levels of interleukin 35 in patients with rheumatoid arthritis," *Tohoku J. Exp. Med.*, **237(2):** 77-82.

Odegaard, J.I. (2016). "Perinatal Licensing of Thermogenesis by IL-33 and ST2," *Cell*, **166(4):** 841-854, doi: 10.1016/j.cell.2016.06.040.

Okragly, A.J. *et al.*, (2016). "Elevated levels of Interleukin (IL)-33 induce bone pathology but absence of IL-33 does not negatively impact normal bone homeostasis," *Cytokine*, **79:** 66-73. doi: 10.1016/j.cyto.2015.12.011.

Okragly, A.J. (2016). "Elevated levels of Interleukin (IL)-33 induce bone pathology but absence of IL-33 does not negatively impact normal bone homeostasis," *Cytokine*, **79:** 66-73.

Pinto, M.R., A. Kakehasi and M.V Andrade (2017). "FRI0112 Serum levels of IL-33 and SST2 are associated with functional disability in rheumatoid arthritis." B.M.J. Publishing Group Ltd.

Su, L.C., X.Y. Liu, A.F. Huang and W.D. Xu (2018). "Emerging role of IL-35 in inflammatory autoimmune diseases," *Autoimmun. Rev.*, **17(7):** 665-673.

Salama, A.A., A.B. Mahmoud, D.R. Al-Sharaki and A.F. Gomah (2017). "Role of interleukin-33 in rheumatoid arthritis patients from Menoufia University Hospitals," *Menoufia Med. J.*, **30(3):** 952.

Talabot-Ayer, D., C. Lamacchia, C. Gabay and G. Palmer (2009). "Interleukin-33 is biologically active independently of caspase-1 cleavage," *J. Biol. Chem.*, **284(29):** 19420-19426.

Veeraveedu, P.T. (2017). "Ablation of IL-33 gene exacerbate myocardial remodeling in mice with heart failure induced by mechanical stress," *Biochem. Pharmacol.*, **138:** 73-80.