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ORIGINAL ARTICLE

Assessment of Forkhead BoxP3 (Foxp3) Gene Expression in Rheumatoid Arthritis

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ABSTRACT:

Background: Rheumatoid arthritis (RA) is a common autoimmune inflammatory condition that particularly impacts joints. It is featured by an intensifying inflammation symmetrically regards the impacted joints causing destruction of cartilage and bone, disability and limitation of movement. The study aimed to determine gene expression of Forkhead box P3 (FoxP3) among rheumatoid arthritis patients. In addition, to assess disease modifying antirheumatic drugs (DMARDs) effect on FoxP3 expression.

Methods: This case-control study included 66 subjects allocated equally into: Group (1) included RA patients who were DMARDs naïve, Group (2) included 22 RA cases who were DMARDs treated, and Group (3) included 22 apparently healthy volunteers. All patients of the studied groups were conducted to history taking, complete physical and clinical assessment, Evaluation of disease activity by DAS-28, laboratory tests, and FoxP3 gene expression

Results: FoxP3 expression levels decreased among RA cases compared to controls. The lowest levels were detected among DMARD naïve group. There was statistically substantial negative association between FoxP3 expression values with TJC, SJC, GH, DAS-28 score, ESR, CRP, and RF titer among the studied RA patients. There was a remarkable negative relationship between FoxP3 expression levels with disease duration among the treated RA cases (P=0.04).

Conclusions: There are substantial differences of Forkhead box P3 (FoxP3) gene expressions between RA cases and normal controls. Moreover, the expression levels of these genes are affected by DMARDs treatment among RA cases and negatively associated with the disease activity.

Keywords: FoxP3 ; RAD ; AS-28 ; real-time PCR.

INTRODUCTION

Rheumatoid arthritis (RA) is a common autoimmune inflammatory condition that particularly impacts joints. It is featured by an intensifying inflammation symmetrically regards the impacted joints causing destruction of cartilage and bone, disability and limitation of movement. In early stages, few joints are distressed while in advanced stages most of joints are impacted and there are other symptoms in the articulation all over the body. The prevalence of the disease is 0.4 to 1.3 affected by age, sex and place as RA is higher 2-3 times in females than males, it is more incident in the 6th decade of life and is decreasing in rural than urban [1].

Clinical symptoms of early stages of RA are different from later stages or incomplete treated disease stages. In early stages RA is featured by symptoms like fatigue, morning stiffness, tender and swollen joints, and laboratory investigations characterized by increased C-reactive protein (CRP) values and an elevated erythrocyte sedimentation rate (ESR). In later stages or incompletely treated RA there are serious systemic manifestations in addition to progressive joint manifestations and all these symptoms lead to increase mortality [2].

Like many autoimmune diseases, the causative factors of RA are multifactorial. These factors may be genetic, environmental or inflammatory. About 50% of RA risk is contributed to genetic

predisposition. There are many environmental factors like smoking and infections that trigger RA. Also, family history, old age and female sex are risk factors for RA. RA is characterized by inflammatory pathway in which there is over production of TNF and interleukin 6 that cause the destructive process [3].

The FoxP3 (Forkhead box P3 gene) has an important involvement in function of Tregs cells (T regulatory cells), preservation of immunological tolerance and response control. Also, this transcription factor is crucial for the production of the Treg phenotype. FoxP3 's strong inhibitory T-cell stimulation and effector function may contribute to the immunopathology of autoimmune conditions and cancer. Foxp3 and Treg cells had a role in induction of autotolerance and immune homeostasis and Foxp3 is the main regulator of their function and progression. Foxp3 gene is located on chromosome Xp11.23 So, it has been named as a distinctive gene in pathogenesis of RA [4,5].

Disease-modifying anti-rheumatic drugs (DMARDs) are a class of drugs employed for the RA management. These medications, which are immunosuppressants, can cause RA to remit and delay the disease's course by slowing down joint deterioration. They may also be employed for managing other autoimmune conditions like systemic lupus erythematosus, inflammatory bowel disease, and scleroderma. Methotrexate, hydroxychloroquine, leflunomide, and sulfasalazine are examples of conventional DMARDs [6]. For the greatest RA control, DMARDs can be administered alone or in combination with other DMARDs or medications. Additionally, a particular class of DMARDs called as "biological DMARDs" is accessible. These prevent particular compounds from entering the bloodstream and joints that cause inflammation, and this group is prescribed if traditional DMARDS have not worked [7].

This work aim was to detect gene expression of Forkhead box P3 (FoxP3) among RA cases. In addition, to assess the impact of DMARDs on the expression of FoxP3 and its association with the disease activity.

METHODS

Patients:

This case-control study was performed in Medical Biochemistry and Molecular Biology and Rheumatology and Rehabilitation Departments, Faculty of Medicine, Zagazig University. Sixty-six participants were selected in this study; they were allocated equally into 3 groups:

DMARD-naïve rheumatoid arthritis group (group 1): Twenty-two rheumatoid arthritis cases have not started treatment by DMARDs. DMARD-treated rheumatoid arthritis group (group 2): Twenty-two rheumatoid arthritis cases have been treated by DMARDs for at least 3 months. Control group (group 3): Twenty-two apparently healthy, age- and sex-matched subjects were included as a control group. They were chosen to be apparently and clinically free from any disease and were not receiving any drugs.

The study was conducted after obtaining approval from Institutional Review Board (IRB #10567/12-3-2023) and written informed consent from all cases. The research was conducted under the World Medical Association's Code of Ethics (Helsinki Declaration) for human research.

Cases with the following criteria were included; Patients diagnosed by rheumatoid arthritis are classified according to the criteria established in 2010 by ACR/EULAR [8]. Seropositive RA cases (who had positive rheumatoid factor). Cases with age > 20.

Cases with the following characteristics were excluded; Refusal of the patient to give consent and lack of cooperation. Women who are pregnant or breastfeeding. Presence of other diseases that may interfere with the study parameters like severe infection and malignancy.

Methods:

All patients were conducted to full history taking, complete physical and clinical assessment, Evaluation of disease activity by the modified version of the 28-joint Disease Activity Score (DAS28) [9]. Laboratory tests (Rheumatoid factor (RF), anti-cyclic citrullinated peptide antibody (Anti CCP Ab), ESR, CBC and CRP).

Disease Activity (DAS28) assessment: The visual analogue scale (VAS) and ESR were employed to assess global health and disease activity, as well as the number of sore and swollen joints (28 joints total) [9]. The disease activity level can be elucidated regarding to DAS28 as remission (DAS28 < 2.6), low (2.6 ≤DAS 28<3.2), moderate (3.2≤DAS28≤5.1), or high (DAS28 >5.1) [10].

Sample collection: Ten milliliters (10 mL) of venous blood were collected in EDTA tubes under complete aseptic condition, divided as follows: two ethylene diamine tetra acetic acid (EDTA) tubes; one for CBC and the second for Polymerase Chain Reaction (PCR) analysis, a citrated tube for ESR

and a plain tube was used for serum separation for assay of CRP and anti-CCP and RF.

Specimen storage: Whole blood specimens anti-coagulated and fresh were stored at 15-30°C.The sample for molecular workup was stored at -20°C. Hemolyzed serum samples were discarded, and repeated freezing and thawing was avoided.

Specimen processing:200 µl of whole fresh blood sample was used for RNA extraction. The extract will be stored at temperature (-20°C) for further use after collection of all samples of RNA extracts (2 months) to be employed in PCR.

Total RNA extraction from blood: Briefly, total RNA was extracted from the blood using Trizol (Invitrogen; Thermo Fisher Scientific, Inc.) according to manufacturer instructions. For assessing the quality of RNA, the A260/A280 ratio was examined utilizing the NanoDrop® ND–1000 Spectrophotometer (NanoDrop Technologies; Wilmington, Delaware, United States) for 1.5 µl od the RNA. For cDNA formation, a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems™, USA) was used.

Real-time quantitative PCR (RT-qPCR) analysis: RT-qPCR was performed in a Rotor-Gene Q 2plex RT PCR System (Qiagen, Germany) using TOPreal™ qPCR 2X PreMIX (SYBR Green with low ROX) (Enzynomics, Korea) regarding the manufacturer's instructions. FoxP3 forward 5' TCTTCCTTGAACCCCATGCC 3', and reverse 3' AAATGTGGCCTGTCCTGGAG 5', and GAPDH forward 5' GACAGTCAGCCGCATCTTCT 3', and reverse 5' GCGCCCAATACGACCAAATC 3'.

The PCR cycling circumstances included primary denaturation at 95°C for 12 minutes followed by 40 cycles of denaturation at 95°C for 20 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds. The primers were synthesized by Sangon Biotech (Beijing, China). The expression level of the target genes was normalized employing the mRNA expression of GAPDH. The findings are presented as fold-changes compared to the control group utilizing the ($2^{-\Delta\Delta Ct}$) method [11].

Statistical Analysis:

All data were analyzed employing SPSS 26.0 for windows (SPSS Inc., Chicago, IL, USA). The mean ± SD, interquartile range, and range were used to convey quantitative data, while absolute frequencies (number) and relative frequencies (%) were used to express qualitative data. When comparing more than 2 groups of normally distributed data, the ANOVA test was employed. For relation between quantitative variables of 2 groups, independent t-test (Parametric test) used for comparison of two independent means and two samples when the variables are quantitative, randomly selected & normally distributed. Mann-Whitney U test (Nonparametric test) utilized to compare outcomes between two independent groups as comparing the medians between the two populations. Kruskal-Wallis test was employed for comparing two or more independent samples of equal or different sample sizes that is not normally distributed. For correlation between two quantitative variables, Pearson's correlation employed for parametric normally distributed data. Spearman's rank correlation test employed for ordinal data or if the assumptions of normality of data not satisfied. A helpful method for assessing the sensitivity and specificity of quantitative diagnostic tests that divide cases into two groups is to utilize ROC curve. Data with P<0.05 considered significant

RESULTS

As regards disease duration among RA patients, there was a highly substantial variance between groups 1 and 2 as disease duration was substantially increased among group 2 patients (P <0.001). As regards laboratory data among studied groups, there was noticeable variation between groups as regards ESR and CRP levels, where Group 1 had significantly the highest level, followed by Group 2, while Group 3 had significantly the lowest levels. As regards RF and Anti-CCP titers, there was no remarkable variance between Group 1 and Group 2 (P >0.05), however both groups had remarkably higher values in comparison to Group 3 (P <0.05). There was a highly substantial variance between the three groups as regards FoxP3 expression, as FoxP3 expression was significantly the highest among Group 3, followed by Group 2 and the lowest among Group 1 (P <0.001). (Table 1)

On comparing clinical data among the studied RA groups, Group 1 had significantly higher SJC and TJC than Group 2 (P <0.05). Also, group 1 exhibited substantially elevated GH and DAS-28 scores in comparison to Group 2 (P <0.001). Most of RA cases in group 1 and 2 had moderate disease activity while high disease activity represented 45.5% of group 1 and 0% of group 2. All the cases in group 2 were on DMARDs, while none of the patients in group 1 were on DMARDs (P<0.001). (Table 2)

There was a substantial inverse relationship between FoxP3 expression levels with TJC, SJC, GH, DAS-28 score, ESR, CRP, and RF titer among the studied RA cases (P <0.05). (P <0.001). (Table 3)

There was no remarkable relationship between gene expression and type of therapy among the RA patients (P>0.05). (Table 4)

There was a substantial inverse relationship between FoxP3 expression levels with disease duration among the treated RA patients (P=0.04). (Table 5)

On conducting ROC (Receiver operating characteristic) curve to discriminate RA cases from healthy controls, FoxP3 expression shows the highest sensitivity (95.5%) and specificity (86.4%) at cut-off point 0.82 with AUC 0.957, so FoxP3 expression could be considered as an excellent biomarker in discriminating RA patients from healthy controls. (Table 6), Fig(1)

Table (1): Demographic data, laboratory tests, and gene expression among the studied groups

	RA patients		Controls	Test	p
	Group 1 (n=22)	Group 2 (n=22)	Group 3 (n=22)		
Age (years) *Mean±SD* *Range*	 46.2±12.9 (25 – 65)	 46.9±11.1 (25 – 64)	 45±13.8 (25 – 66)	F 41.6	0.9
Sex (N. %) Female Male	 17 (77.3%) 5 (22.7%)	 19 (86.4%) 3 (13.6%)	 15 (68.2%) 7 (31.8%)	X^2 2.07	0.36
Duration (months) *Median (IQR)* *(range)*	 0.5 (0.44) (0.5 – 1)	 9.5 (10.5) (3 – 36)	 -	MW	<0.001
ESR *Median (IQR)* *(range)*	 20 (12.75) (11 – 52)	 12.5 (7.5) (7 – 28)	 6.5 (4) (1 – 12)	 KW	P^1=0.004 P^2<0.001 P^3<0.001
CRP *Median (IQR)* *(range)*	 12.5 (6.8) (4.5 – 42.1)	 5.5 (6.4) (1.05 – 24.3)	 3.85 (2.1) (1.2 – 5.1)	 KW	P^1=0.004 P^2<0.001 P^3=0.009
Negative (n. %) *Positive (n. %)*	2 (9.1%) 20 (90.9%)	14 (63.6%) 8 (36.4%)	22 (100%) 0 (0%)	X^2	<0.001
RF titer *Median (IQR)* *(range)*	 42.8 (37.25) (17.3 – 152)	 31.45 (41.03) (16.5 – 113)	 8.1 (3.95) (2.7 – 12)	 KW	P^1=0.788 P^2<0.001 P^3<0.001
Negative (n. %) *Positive (n. %)*	0 (0%) 22 (100%)	0 (0%) 22 (100%)	22 (100%) 0 (0%)	X^2	P <0.001
Anti-CCP titer *Median (IQR)* *Range*	 37.3 (50.68) (17.1 – 496.5)	 31.5 (43.2) (21.6 – 226)	 8.3 (3.5) (5.7 – 13)	 KW	P^1=0.997 P^2<0.001 P^3<0.001
Negative (n. %) *Positive (n. %)*	3 (13.6%) 19 (86.4%)	0 (0%) 22 (100%)	22 (100%) 0 (0%)	X^2	P <0.001
FOXP *Median (IQR)* *(range)*	 0.19 (0.11) (0.10 – 0.37)	 0.69 (0.26) (0.42 – 1.04)	 1.07 (0.81) (0.80 – 8.52)	 KW	P^1<0.001 P^2<0.001 P^3<0.001

F: ANOVA test of significance,X^2: chi square test, MW: Mann-Whitney U Test, *KW: Kruskal-Willis Test, P^1: Comparison between Group 1 & Group 2, P^2: Comparison between Group 1& Group 3, P^3: Comparison between Group 2 & Group 3, P-value >0.05: Insignificant, P-value ≤0.05: Significant, P-value <0.001: Highly significant P-value ≤0.05: Significant, SD: Standard deviation, IQR: Interquartile range

Table (2): Clinical data among RA patients				
	RA patients		**Test***	**p**
	Group 1 (n=22)	**Group 2 (n=22)**		
TJC *Median (IQR)* *(range)*	6 (5.75) (2 – 16)	2 (2) (0 – 10)	MW 100	<0.001
SJC *Median (IQR)* *(range)*	6 (4.75) (0 – 16)	2.5 (2) (0 – 8)	MW 120.5	0.004
GH *Mean±SD* *(range)*	55.45±20.41 (30 – 100)	35.45±15.03 (10 – 60)	t 3.70	<0.001
DAS-28 score *Mean±SD* *Range*	5.01±0.90 (3.67 - 6.88)	3.78±0.92 (2.08 – 5.10)	t 4.49	<0.001
DAS-28 grades No Mild Moderate High	0 (0%) 0 (0%) 12 (54.5%) 10 (45.5%)	2 (9.1%) 6 (27.3%) 14 (63.6%) 0 (0%)	X^2	<0.001
Treatment Corticosteroids DMARDs Monotherapy combination therapy Triple therapy	7 (31.8%) 0 (0%) - - -	5 (22.7%) 22 (100%) 0 (%) 16 (72.7%) 6 (27.3%)	X^2	0.50 <0.001 - - -

*MW: Mann-Whitney U Test, X^2: chi square test (Fisher-exact test), t: Independent sample t-test
P-value >0.05: Insignificant, P-value ≤0.05: Significant, P-value <0.001: Highly significant
TJC: tender joint count, SJC: swollen joint count, GH: General Health, DAS-28: Disease activity index, SD: Standard deviation, IQR: Interquartile range
Monotherapy: methotrexate only, combination therapy: two DMARDs of methotrexate, leflunomide, sulfasalazine, and hydroxychloroquine. Triple therapy: three DMARDs of methotrexate, leflunomide, sulfasalazine, and hydroxychloroquine.

Table (3): Correlation between Foxp3expression and different patients' parameters		
	FOXP expression	
	r	*P-value*
Age (years)	-0.054	0.668
TJC	-0.373	0.002
SJC	-0.358	0.003
GH	-0.433	<0.001
DAS-28 score	-0.466	<0.001
ESR	-0.353	0.004
CRP	-0.278	0.024
RF titer	-0.292	0.017
Anti-CCP titer	-0.181	0.145

TJC: Tender Joint Count, **SJC:** Swollen Joint Count, **GH:** General Health, **DAS-28:** Disease Activity Index, **ESR**: Erythrocyte Sedimentation Rate, **CRP:** C-Reactive Protein**, RF titer:** Rheumatoid Factor, **CCP titer:** Cyclic Citrullinated Peptide Antibody

Table (4): Gene expression analysis according to therapy among RA patients

	Combination therapy (n=16)	**Triple therapy (n=6)**	**Test***	**p**
FOXP *Mean ± SD* *(range)*	 0.69± 0.14 (0.52 – 1.00)	 0.82± 0.24 (0.42 – 1.04)	 t	0.13

*t: Independent sample t-test, P-value >0.05: Insignificant, P-value ≤0.05: Significant, P-value <0.001: Highly significant

Foxp3: Forkhead boxP3,

Table (5): Correlation between Foxp3 expression and different patients' parameters among the treated group

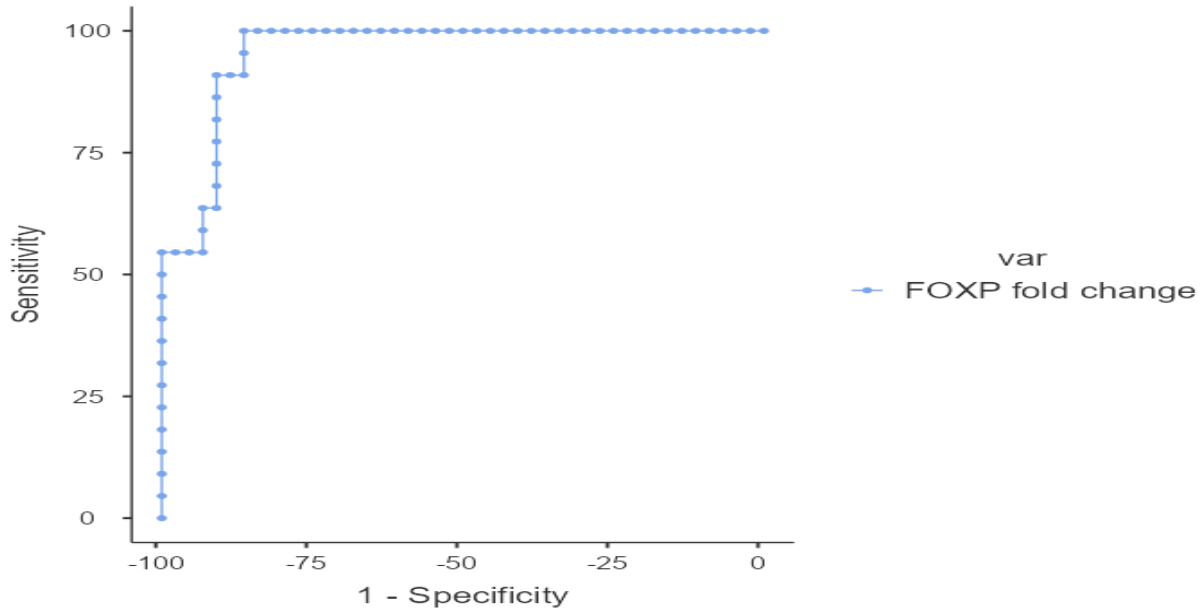
	FOXP expression	
	r	***P-value***
Age (years)	-0.233	0.297
Duration	-0.436	0.04
TJC	0.034	0.971
SJC	0.048	0.833
GH	0.033	0.885
DAS-28 score	0.190	0.397
ESR	0.241	0.126
CRP	0.316	0.152
RF titer	-0.011	0.962
Anti-CCP titer	-0.033	0.883

TJC: Tender Joint Count, SJC: Swollen Joint Count, GH: General Health, DAS-28: Disease Activity Index, ESR: Erythrocyte Sedimentation Rate, CRP: C-Reactive Protein, RF titer: Rheumatoid Factor, CCP titer: cyclic citrullinated peptide antibody

Table (6): ROC curve analysis of Foxp3 gene expression in discriminating between RA patients and Controls

	Cut-off	Sensitivity	Specificity	PPV	NPV	AUC
FOXP	0.82	95.5%	86.4%	77.78%	97.44%	0.957

FOXP: Forkhead boxP3



Fig(1): ROC curve analysis of FOXP3 gene expression in discriminating between RA patients and Controls

DISCUSSION

The most significant factors contributing to RA include angiogenesis, fibrosis and hyperplasia of synovial cells, and the breakdown of bone and cartilage. Although RA is not a fatal condition in and of itself, some people may experience years of reduced survival due to its consequences. Joint discomfort and impairment are widespread in day-to-day living. Cases' quality of life may be significantly impacted by distorted joints and the difficulty or impossibility of doing even routine duties. When given the chance, CD4 T cells with a naive phenotype that are activated by antigen-presenting cells (APCs) can develop into one of many distinct cell lineages. These cells' dysregulation and atypical numbers could lead to inappropriate humoral and cellular immune responses [12].

Therefore, the objective of this study was to detect gene expression of FoxP3 among RA cases.

Our case control study included 66 subjects aalocated into: group (1) included 22 RA patients who were DMARDs naïve, 17 of them were females (77.3%) and 5 were males (22.7%), there ages mean age was 46.2± 12.9 years (25-65). The second group included 22 RA patients who were DMARDs treated (group 2), 19 of them were females (86.4%) and 3 were males (13.9%), their mean age was 46.9±11.1 years (25-64). The third group (group 3) included 22 subjects who were apparently healthy volunteers. 15 (68.2%) of them were females and 7 (31.8%) were males. Their mean age was 4513.8 (25-66).

Ikram et al. [13] assessed the prevalence of -924A/G and -3279C/A polymorphisms in the Foxp3 gene's promoter region in Egyptian RA patients versus normal participants. The studied population mean ages and range were 45.2, 24 – 60 years and 43.8, 25 – 62 years, for patients and control subjects, respectively.

Al-Jumaily et al. [14] investigated The study examined the possible diagnostic value of high values of IL-10 and FoxP3 gene expression in RA cases. By looking at age and gender, the demographic distribution of RA cases (n = 60) and control (n = 30) was analyzed. They proposed that women made up a substantial portion of the cases population, accounting for 75% (45) of the total sample size, while men made up 25%. Likewise, there was a matched participant distribution in the control group (15 men and 15 women). Additionally, RA was more common in those between the ages of 51 and 71, and it was more common in women than in men.

This result could be due to that the One of the many chronic autoimmune disorders that primarily affect women is RA. There are two to three times as many female cases as male cases. Uncertainty surrounds the precise processes underlying the female preponderance of RA. One clear explanation is that estrogens influence immunological function, according to some evidence [15].

As regards disease duration among RA patients, there was a highly substantial variation between Group 1 (median, 0.5 month) and Group 2 (median,

9.5 months) as disease duration was substantially elevated among group 2 cases (P <0.001).

On comparing clinical presentation among the studied RA groups, Group 1 had significantly higher TJC (median, 6) and SJC (median, 6) in comparison to Group 2 (median, 2 and 2.5, respectively) (p<0.05) In the investigation of Kim et al., [16] the mean ± SD of tender joint count was 7.9 ± 6.6 and the mean of swollen joint count was 4.6 ± 6.3.

In our study, Group 1 had a substantially elevated DAS-28 score compared to Group 2 and most of RA cases in group 1 and 2 had moderate disease activity while high disease activity represented 45.5% of group 1 and 0% of group 2. All the cases in group 2 were on DMARDs, while none of the patients in group 1 were on DMARDs.

Boyd et al. [17] reported that DAS28 score was highest at the initial visit where the cases were still untreated, and following the start of therapy, the score declined over time, suggesting both a reduction in joint inflammation and an improvement in functional capacity.

As regards laboratory data among studied groups, there was remarkable variance between the three groups concerning ESR and CRP levels, where Group 1 had the highest levels (median, 20 and 12.5, respectively), followed by Group 2 (median, 12.5 and 5.5, respectively), while Group 3 (median, 6.5 and 3.85, respectively) had the lowest levels. Cribbs et al. [18] have shown that after 6 months of DMARD therapy, RA patients have decreased levels of ESR, CRP and DAS28.

Ikram et al. [13] showed that ESR was elevated in all patients and CRP was positive in 60% but in our study, CRP was positive in about 90% of group 1 and 30% of group 2.

As regards RF and Anti-CCP titers, there was no remarkable variance between Group 1 (median, 42.8 and 37.3, respectively) and Group 2 (median, 31.45 and 31.5, respectively) (P >0.05), however, both groups had substantially elevated values compared to Group 3 (P <0.05). Also, Al-Saadany et al. [19] found that in RA cases, laboratory parameters including ESR, CRP, RF and anti-CCP levels were substantially elevated in RA cases compared to controls.

In the present study, there was a highly substantial variation between the groups concerning FoxP3 expression, as FoxP3 expression was significantly the highest among Group 3, followed by Group 2 and the lowest levels were among Group 1 (P <0.001). Also, Patel et al. [20] reported Foxp3 gene expression fold substantially declined (P≤0.01) in RA cases and Al-Jumaily et al. [14] found a substantial decline (P≤0.01) in Foxp3 gene expression in RA cases compared to healthy participants and concluded that arthritis patients may benefit from FoxP3 gene expression test. Moreover, lower mean Foxp3 levels were reported by Gaafar et al. [21] in RA patients compared to controls (16.9±6.16 and 26.4±14 respectively p=0.008) and (1.01±0.87 and 1.72 ± 1.27 respectively p<0.001).

Nevertheless, Ryder et al., [22] found that RA cases had higher levels of full-length FoxP3 mRNA expression than healthy controls. Additionally, Paradowska-Gorycka et al. [23] reported an elevated serum Foxp3 is higher in cases than control group (51% vs 18%, respectively), and Ikram et al. [13] found that Foxp3 serum levels were remarkably elevated among RA group compared to the control group (mean ± SD = 5.52 ± 3.31 and 4.03 ± 1.19, respectively, P=0.01).

Moreover, findings in our study revealed significant inverse association between Foxp3 expression levels with TJC, SJC, GH, DAS-28 score, ESR, CRP, and RF titer among the studied RA patients (P <0.05). Also, Kanjana et al. [24] demonstrated that Patients with remission have a greater level of Foxp3+ Treg inhibitory activity than those with active RA. Additionally, in individuals with moderate to severe disease, it has an inverse relationship with the disease activity score-28. Furthermore, in remission as opposed to the active state, there are more Foxp3+ Treg cells and a greater Foxp3+ Treg ratio. When combined, Foxp3+ Treg inhibitory activity shows that the immune system imbalance has been corrected in cases with active RA, which makes it a possible prognostic indicator and indicator of immunologic remission in RA.

On the other hand, Ju et al. [25] suggested that Foxp3+ T cells were positively associated with the DAS28-ESR (P=0.042), antiCCP titer (P=0.049), swollen joint counts (P=0.046), VAS scores (P=0.037), and no association was detected between Foxp3+ T cells and tender joint counts. Also, Ikram et al. [13] found that Foxp3 serum level was substantially associated to disease activity as presented by DAS28 score and grade. Also, it was significantly correlated to both disease duration and number of swollen joints. There was a remarkable increase in Foxp3 levels among cases who tested positive for CRP, RF and ACCP. They indicated that In individuals with RA, the blood level of

Foxp3 is not a good predictor of Treg-mediated immune modulation.

However, FoxP3 expression levels were not correlated with DAS28 among the treated RA patients which was in line with Paradowska-Gorycka et al. [23] who stated that serum concentrations of FoxP3 had no significant correlation with disease activity.

In the current study, receiver operation curve (ROC curve) was used to discriminate RA patients from healthy controls, analysis showed that FoxP3 expression shows highest sensitivity (95.5%) and specificity (86.4%) at cut-off point 0.82 with AUC 0.957, so FoxP3 expression could be considered as an excellent biomarker in discriminating RA patients from healthy controls.

We admit that this experiment had certain limitations, even if some of our data were statistically significant. The sample size was quite small. Second, no cases from other regions of Egypt were enrolled; they were exclusively selected from Zagazig University Hospitals. Third, ethnic differences could be significant factors influencing this kind of genetic research. Fourth, RA is a complicated illness with possible gene-gene and gene-environment interactions. Therefore, the genetic component of RA cannot be fully explained by the expression levels of two genes alone.

Conclusion

There was substantial variance of FoxP3 gene expression between RA cases and healthy controls. Moreover, the expression level of FoxP3 was affected by DMARDs treatment among RA cases. The evaluation of the disease activity of this autoimmune disease and response to treatment are important for medication interventions, while the detection of this gene expression level could serve to ascertain the extent of condition activity while patients are treated with DMARDs. Further investigation with increased sample size is needed to obtain more generalized findings.

Conflict of interest: None.

Financial Disclosures: None.

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