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Correlation between Anti-infliximab and Anti-CCP Antibodies Development in Patients with Rheumatoid Arthritis Treated with Infliximab in Baghdad Teaching Hospital

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Summary:
Background: *Many of patients with rheumatoid arthritis was currently successfully treated with infliximab(anti-tumor necrosis factor);however, about 30% of the patients do not responded to infliximab.one of postulated hypotheses of not responding is the fast clearance of infliximab due to development of infliximab-anti-infliximab complexes.*
Objective: *to study the correlation between anti CCP and anti-infliximab antibodies in patients with rheumatoid arthritis treated with infliximab.*
Patients and Methods: *fifty Iraqi RA patients(36 females and 14 males) compared with 50 control(25 healthy control and 25 case control (patients with RA on other treatment)) were enrolled in this study from begging of March 2014 till end of September 2014.All patients were diagnosed by full history, complete clinical examination and laboratory test. Anti-infliximab and anti-CCP antibodies were measured by using enzyme-linked immunosorbent assay in serum of Iraqi patients with RA treated with infliximab for more than 3 months duration.*
Results: *among fifty patients with RA on infliximab treatment for more than three months duration were included in this study, infliximab antibodies were detected in 35(70.0%),anti-CCP were detected in 47(94.0%),anti-infliximab and anti-CCP antibodies were not reported in 15(30.0%, 3(6.0%) respectively .out of 47(94.0%) of patients were positive for anti-CCP;35 (74.5%) were positive for anti-infliximab, while other 12 (25.5%)patients were negative. From total 50 patients were investigated; three of them were negative for both anti-infliximab and anti-CCP anti-bodies.A strong correlation between anti-CCP and anti-infliximab antibodies was observed (P-value<0.01) in patients with rheumatoid arthritis after treatment with infliximab.*
Conclusion: *the study shown thatRA patients with anti-CCP positive more susceptible to develop anti-infliximab antibodies in their serum after infliximab therapy.*
Key words: *anti-infliximab, anti-CCP (anti-cyclic-citrullinated peptide) antibodies, Rheumatoid arthritis.*

I. Introduction:

Rheumatoid arthritis (RA) is a mostcommon, chronic,disabling, and autoimmune inflammatory disease that is characterized bysignificant pain, progressive joint disorder and functional disability (1). Its prevalence was estimated at 0.5 - 1.0 percent of adults worldwide (2, 3), while in Iraqi populations was reported in around 1 %(4).

Symmetric highly inflammatory polyarthritis of peripheral joints is the hallmark of the disease (5). The condition is also systemic in that it often affects many extra-articular tissues throughout the body, including skin, blood vessels, heart, lungs, and muscles. The gradual involvement of multiple joints into pathophysiological process eventually results in articular destruction, ensuing instability, deformity and collateral pain. As the pathology progresses, chronic pain and functional disability dominates one's life and lessens everyday enjoyment and comforts (6).Approximately 10-15% of patients was remained with active and progressive disease resistant to conventional therapies and required for anti-tumor necrosis factor-alpha (TNF-α) at some time during the course of their disease (7).

At present, the biological drug, infliximab had been demonstrated efficacy against RA structural involvement and clinical activity, but its treatment with infliximab had been associated with development of anti-bodies against this biologic (8, 9).The RA is an inflammatory and autoimmune rheumatism associated with

numerous autoantibodies, one of the most important and routinely used for diagnosis of RA: anti- cyclic citrullinated peptide (anti-CCP),the presence of this antibody was leaded for diagnosis of >99% of patients with RA (10, 11).

Together with the classical clinical features of the disease, serological abnormalities, themost important one, anti-cyclic citrullinatedpeptide antibodies (anti-CCP) have been shown to be usefuldiagnostic tools—particularly in the early stages of thedisease—and to be predictive of disease progression andradiological damage(12). In particular, anti-CCP seems topossess a strong specificity for rheumatoid arthritis, thoughthis was accompanied by a relatively poor sensitivity (13).

However, the effect of TNF-α blocker on autoantibodies associated with RA had not been clearly proven because of conflicting results; infliximab is the agent that has been most studies in pivotal studies(14).this autoimmunity induced by TNF-α was observed and more pronounced with infliximab(15).

To the best of our knowledge, no reported study on Iraqi patients with RA had been simultaneously analyzed the association between anti-infliximab and anti-CCP antibodies after treatment with infliximab.

II. Patients and methods:

Patients: Fifty patients (36 females and 14 males),their mean age (45.3) years who attended to medical city, Baghdad teaching hospital, Department of Rheumatology (Biological therapy unit) were included in this study during periodFrom begging of March 2014 till end of September 2014, all patients were treated with biological agent (intravenous infusion of Infliximab of 3 mg/kg at baseline, and at 2 and 6 weeks then every 8 weeks), for at least three months duration, The patients werecompared to 50 control group (25 patients with RA on other treatment and 25 healthy individuals from central blood bank) who were randomly selected as a control groups, written informed consents for the research were obtained from all the enrolled patients and controls.

All these patients met the revised criteria for RA (the American College of Rheumatology 1987) (16) and their disease activity were assessed by using Disease Activity Score in 28 joints (17).

Methods: Two ml of blood were aspirated from each individual and left to clot at room temperature ,then centrifuged and serum was collected in aliquots to store in(- 18C) until needed for investigation of anti-CCP and anti- infliximab antibodies.

Kits and reagents: human anti infliximab antibody ELISA kit (*Matriks Biotek*,Germany), human anti-CCP antibody ELISA kit ((AESKULISA CCP, Germany).

Statistical analysis: statistical analysis was done using SPSS version computer software. T test was used to analyze the data, and calculation of mean difference, Fisher>s exact and Chi-square test for comparison of proportion, P-value of less than 0.05 was considered as statistically significant-value <0.01 as highly significant and P-value <0.001 as extremely significant.

III. Results:

Total of 50 patients (36(72%) females and 14(28%) males) who were enrolled in this study, received treatment of infliximab for more than three months (figure 1).

Fifty patients with RA (36 females and 14 males) their mean age (45.24±9.15) years, and 50 control group (25 healthy and 25 case control), 32females and 18males, their mean age (42.22±8.23) years were included in this study, table 1.

The mean age of patients group did not differ significantly from controls group (45.24. ±9.15years vs 42.22 ±8.23 years, P>0.05) respectively. Also there was no statistical significant difference in the female to male ratio among patients group and controls (72:28 (75%) vs64:36 (60%) , P>0.05) respectively table 1.

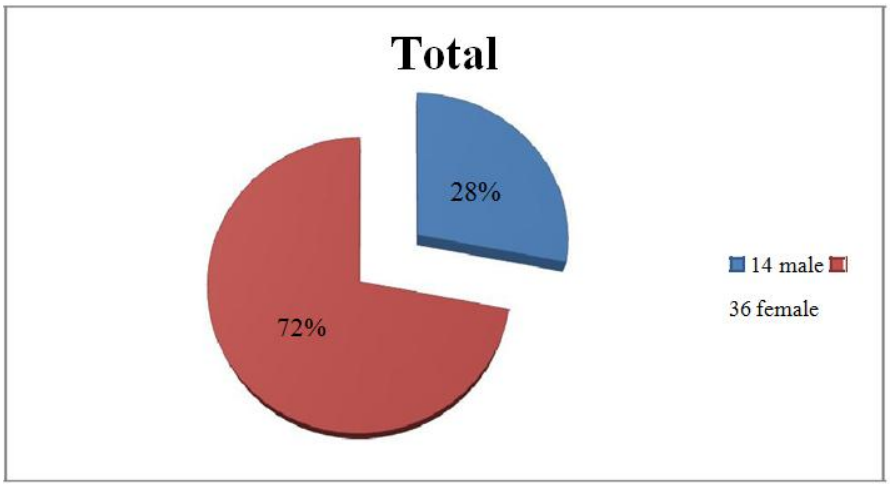

Figure 1: Distribution of patients according to their gender.

Table 1: Demographic characteristic of the case study and control.

Gender	Patients with R.A		Mean age		Controls	Mean age
	No.	%		No.	%	
Male	14	28	41.57±7.51	18	36	40.56±6.53
Female	36	72	46.66±9.16	32	64	43.34±8.13
Total	50	100.0	45.24±9.15	50	100	42.22±8.23

Student t-test = 1.72, P = 0.088

The anti-infliximab anti-bodies were detected in 35(70.0%) patients; (23(65.7%) were females and 13(34.3%) were males), and not detected in 15(30.0%) patients; (13(85.7%) were females and 2(13.3%) were males) with sensitivity (70%) and specificity (100%), while no antibodies were detected in control group, extremely statistical differences(P <0.001)had been found between patients and control group,) but without any statistical difference between male and female,(P-value>.0.05) figure2,table 2.

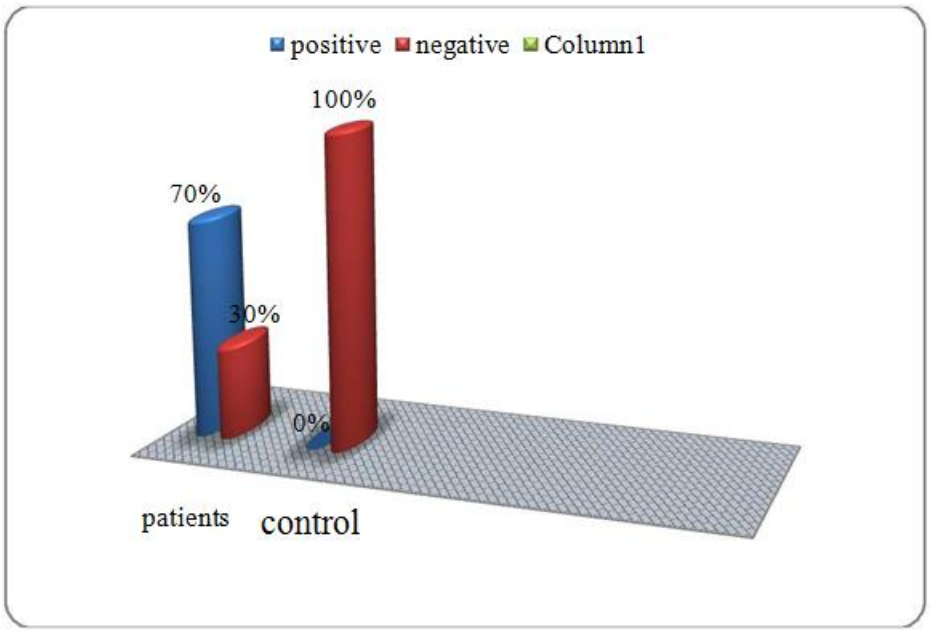

Figure 2: Distribution of serum anti-infliximab antibodies in the case study and control.

X2 = 50.81, P = 0.000001
patients with R.A
control (case and healthy control).
Sensitivity: 70% [55; 82] (95% confidence interval).
Specificity: 100% [91; 100] (95% confidence interval).

Table2: sex distribution of anti-infliximab antibodies in case study.

Gender		Anti-Infliximab				Total
	+Ve	%	-Ve	%	Number	%
Male	12	34.3	2	13.3	14	28.0%
Female	23	65.7	13	86.7	36	72.0%
Total	35	100.0	15	100.0	50	100.0

Chi-square: 2.29
P-value: 0.130506
Sensitivity: 0.34 [0.20; 0.52]
Specificity: 0.87 [0.58; 0.98]
Accuracy: 0.50 [0.36; 0.64]

Out of 50 patients with RA,the anti-CCP antibodies were detected in 47 (94.0%) patients;(14(100.0 %) males and 33(91.7%) females), and were not detected only in 3 (8.3%) female'spatients, out of 50 controls ((25)healthy and(25) case control); the anti-CCP was negative in all healthy controls(100.0%),while, reported positive in 22 (88%) and negative in 3(12%) ofdiseased controls ,with sensitivity (88%) and specificity (100%) with extremely statistical difference (P <0.001) between the patients and control group, but without any statistical difference between male and female(P-value>.0.05)figure 3,table 3.

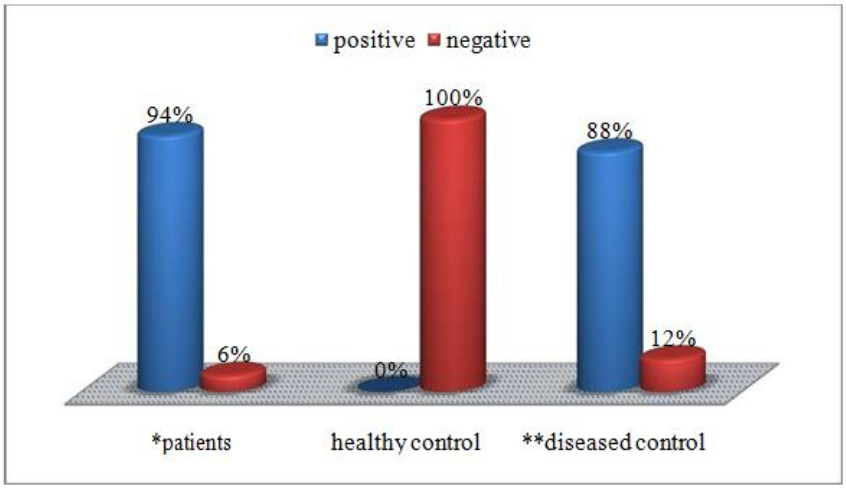

Figure 3: Distribution of serum anti-CCP antibodies in the case study and controls.
* Patients with RA.
**diseased control; patients with RA but not receive infliximab.

Chi-square: 39.29
P-value: 0.0005
Sensitivity: 0.88 [0.68; 0.97]
Specificity: 1.00 [0.83; 1.00]
Accuracy: 0.94 [0.82; 0.98]

Table3: Distribution of anti-CCP antibodiesin the case study according to gender.

Anti-CCP ab		Gender			Total	
	Male	%	Female	%	number	%
+ve	14	100.0	33	91.7	47	94.0
-ve	0	00.0	3	8.3	3	6.0
Total	14	100.0	36	100.0	50	100.0

Chi-square: 1.24
P-value: 0.265252
Sensitivity: 0.30 [0.18; 0.45]
Specificity: 1.00 [0.31; 0.97]
Accuracy: 0.34 [0.22; 0.49]

Out of 47 patients were reported positive for anti-CCP antibodies; 35(74.5%) were positive for anti-infliximab and 12 (25.5%) were negative. Three patients were reported negative for both anti- CCP and anti- infliximab.A strong correlation between anti-CCP and anti-infliximab antibodies was observed (P <0.01) inpatients with rheumatoid arthritis after treatment with infliximab,table 4.

Table 4: anti-infliximab and anti-CCP distribution Crosstab.

Crosstab					
			Anti-infliximab		Total
			+ve	-ve	
CCP	+ve	Count	35	12	47
		% within CCP	74.5%	25.5%	100.0%
	-ve	Count	0	3	3
		% within CCP	.0%	100.0%	100.0%
Total		Count	35	15	50
		% within CCP	70.0%	30.0%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	7.447	1	.006

IV. Discussion:

Rheumatoid arthritis is a common inflammatory disorder manifesting typicallyas a symmetrical polyarthritis, it characterized by chronic inflammation of synovial joints that leads to progressive joints destruction and disability with reduction in quality of life (18).

In our study the female to male ratio was (3:1) and this similar to AL-Rawi et al and alubaidy who found it (3:1) and this probably reflect the sex distribution of Rheumatoid arthritis in our population (4, 11).

The female predominance may be due to hormonal factors such as estrogen which enhances the function of T-helper cells and inhibits the function of T-suppressor cells; also estrogen receptors are present on memory T-cells and on synovial cells (19-21).

The mean age of patients was 45.24±9.15yrs., this is in accordance with other study which mentioned that RA affects usually people who are more than 40 years of age & starts usually after middle age as other AIDs, RA starts after 40 years due to many reasons that lead to depression of the immunity as stress , thymic depression, exposure to different antigens as smoking (tobacco), drugs and chemicals which leads to activation of auto-reactive lymphocytes that interact with self-antigen.(11,22).

The present study revealed anextremestatistical difference of anti-infliximab development between patients and controls in about (70.0%) (P <0.001)withthe sensitivity and specificity((70%&100%) respectively.anti-infliximab anti-bodieswere reported in high percent in females (65.7%) than male (34.3%). these results of our study in line with a study done by Wolbinket al.(43%)in 2006 ,Hoshino et al(35%) in 2012and Krintel et al (54%)in 2013(8,9,23).They were approved that elevated level of the anti-infliximab antibodies are associated with decreasein response of patients to infliximab or reduce of itsefficacy, so increase of failure of treatment of rheumatoid arthritis.

Anti-infliximab anti-bodies neutralize the INF function or increase its clearance from the body by different mechanisms; first, they can inhibit the drug from entering the blood stream.Second, improve the clearance by developing precipitated immune complexes in blood vessels.Third ,increasing splenic clearance. Fourth, inhibit the drug from entering the inflammation sites .Fifth;neutralize its ability to inhibit TNF (24).

The current study had been shown that the anti-CCP was positive in 47 Iraqi patients (94%) with RA($p<0.001$) with sensitivity and specificity (88% &100%)respectivelyas shown in figure3and this was agree with previous study was done by Alubaidy and Al-Ani in 2012 on Iraqi patients with RA after treatment with anti-tumor necrosis factor agents(11,25), and also this is in accordance with other study conducted by Dana in 2007 which mentioned a sensitivity and specificity of (68% & 98%) respectively(26)

ACPA were negative in healthy control group while other study stated that they were positive in 2% of healthy persons, this difference may be due to small sample of our healthy subjects (27).

A strong association was reported by the current study between anti-infliximab and anti-CCP antibodies development in Iraqi patients with RA disease after treatment with infliximab for more than three months duration ($p<0.01$), this result was in a accordance with other study done by Alessandri in 2004 who mention a significant decrease in the titre of anti-CCP in sera of the patients after 24 weeks of anti-TNF-α treatment with infliximab (28).

However, further studies are needed for conformation the effect of anti-CCP anti-bodies on development of anti-infliximab antibodies after treatment with infliximab in patients with RA.

V. Conclusion:

The RA patients with anti-CCP positive more susceptible to develop anti-infliximab antibodies in their serum after treatment with infliximab.

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