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Correlation and Association of serological Parameters with Oxidative and Anti-oxidative stress in Rheumatoid Arthritis patients: An Observational Comparative Study

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Abstract

The aim of our study is to check out the correlation between different biochemical parameters and effect of Oxidative and antioxidative stress on Biochemical parameters in rheumatoid arthritis patients. In this observational comparative study, we collected 30 samples from rheumatoid arthritis patients who have positive RA factor and 30 from control group (with negative RA factor) through random sampling technique. After that we process our samples towards biochemical parameters (Anti-CCP, ANA through ELISA and Latex agglutination) to look into the variation but for correlation we studied the oxidative and anti-oxidative stress through TBARS assay and DPPH assay. Statistical analysis was computed by Fisher's exact test, independent t test and correlation was measured through Spearman rank test. In our sample size the male (n=18), female (n=12) among rheumatoid arthritis patients with age group 21-70 years showed significant correlation with Anti-CCP (p-value <0.001) and TBA (p-value <0.001) but there was no association with ANA (p-value 0.0052) and DPPH (p-value 0.492). As well as gender also showed no association with rheumatoid arthritis (p- value .071). On the basis of our findings rheumatoid arthritis has a significant association with Anti-cyclic citrullinated peptide and Thiobarbituric acid assay. According to our results oxidative stress may be a causative agent of rheumatoid arthritis. So, it is concluded that if anti- oxidative medicine is used as an administrative content for RA patients it will be helpful for the treatment of RA patients.

Keywords: Rheumatoid arthritis, Oxidative stress, Antioxidative stress, Rheumatoid arthritis factor, Inflammation.

Full length article **Corresponding Author*, e-mail: *hina.awais@mlt.uol.edu.pk*

1. Introduction

Arthritis can be acute or chronic as it can lead to inflammation of joints, stiffness of joints, and restricted motion of the joint, and joint disproportion [1]. Rheumatoid arthritis (RA) is an autoimmune disorder in which immune system attacks own body tissues. As the disease progresses from the peripheral joints (fingers, hands, toes, and feet) and then moves to the proximal joints. Although it's a chronic inflammatory condition which affects the bilateral joints like the hands or knees [2]. The pathogenesis of rheumatoid arthritis (RA) involved the following stages: triggering innate immunity and abnormal cytokine networks, maturation initiated at secondary lymphoid tissues or bone marrow, targeting targeting starts with synovitis occurring in small joints, and joint swelling, and fulminant stage is the final stage of the disease and is characterized by severe joint destruction, cartilage damage, and bone erosion [3,4,5,6,7].

The actual cause of RA is not known or clear as there are many theories and hypotheses about RA, including that would be a genetic factor or oxidative stress [8,9]. High levels of reactive oxygen species (ROS) related oxidative stress have been observed in joint inflammation, synovial proliferation, and angiogenesis. However, the presence of reductive stress in CD4+ T cells of RA patients, indicating a different redox characteristic compared to other diseases [10]. The prevalence of RA in Pakistan varies depending on the region and population group. In northern rural population the prevelance rate of RA was 7%, in the poor urban population was 1%, and in affluent urban population was 8% [11]. The most common symptoms of RA include: Joint pain, swelling, redness over joints, synovitis of hands and feet, pain in motion, joint deformity, tiredness, fever and stiffness mostly occurs early in the morning or after sitting for a long time [12]. The diagnosis of rheumatoid arthritis (RA) is typically based on combination of various factors include the patient's symptoms, assessment of risk factors, family history. joint ultrasound sonography, and laboratory markers such as C-reactive protein (CRP) erythrocyte

sedimentation rate (ESR), Rheumatoid factors (RF) antibodies and anti-cyclic citrullinated peptide (anti-CCP), Anti-nuclear antibodies (ANA) in the blood [13]. So, on the basis of these facts the main objective of our study to check the correlation between different biochemical parameters in Rheumatoid Arthritis patients as well as to check out the effect of oxidative stress on biochemical parameters in rheumatoid arthritis patients.

2. Material and Methods

This observational comparative study was directed at The University of Lahore, Pakistan from March 2023 to October 2023 was in accordance with the declaration of World Medical Association (WMA) made at Helsinki (2013). The blood samples (n=30) from the patients of rheumatoid arthritis (RA) and from control group (n=30) were collected from the Combined Military Hospital Lahore and Lahore general Hospital Lahore, through the random sampling technique. To evade the outcome of confounding variables, the inclusion and exclusion criteria base on the RA factor results (either positive or negative) as well as any other physical abnormality and blood disorder.

2.1. Estimation of biochemical parameters

All the procedures were performed under the World Health Organization's standard operating procedures. The serum samples of the patients and control group were examined on Latex Agglutination kit for RA factor and ORGENTEC (Diagnostika GmbH) Anti-CCP ELISA kit as well as CALBIOTECH A.N.A ELISA kit for the estimation of Anti-CCP and ANA.

2.2. Thiobarbituric acid Assay

We took 500 ul serum sample and then we added 500ul Thiobarbituric Acid reagent (sigma-Aldrich) in Eppendorf and incubate at 95 degree in water bath for 10 minutes. After incubation centrifuge the samples for 10 Minutes at 150*10^6 and took the absorbance at 532nm in double beam Spectrophotometer (U-2900 HITACHI).

2.3. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

After the evaluation of biochemical parameters, antioxidant activity was done to inhibit the oxidative stress in the confirmed RA patients' samples by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, USA), sodium phosphate buffer (Honeywell Fluka, USA), and methanol solution (Sigma-Aldrich, USA). The percentage of the DPPH scavenging effect was calculated by the following formula [14]:

$$\text{DPPH scavenging effect} = \frac{A0 - A1}{A0} \times 100$$

A0 = The absorbance of control
A1 = The absorbance of sample

2.4. Data Analysis

The analysis of the data was completed with the help of statistical package for social sciences (IBM SPSS Statistics 25.0 x64) software. Independent t test, Fisher exact test and Spearman's rank correlation, ± min, ± max, ± mean, and ± Std-dev was computed to assess the results [15].

3. Results and discussions

Arthritis can lead to inflammation of joints, joint stiffness, and restricted motion of the joint, and joint disproportion. In arthritis, the cartilage cap is damaged by the uric acid crystals and causes swelling of the joints and severe pain. Rheumatoid arthritis (RA) is a chronic condition mostly caused by the interaction between genes and environmental parameters, including tobacco, that mainly target synovial joints [16].We collected 30 samples of R.A patients in which number of Males was (n=18) and Females (n=12) and 30 samples of Normal Persons which have (n=11 male) and (n=19 female). The minimum age in our concerned population was 21 years while the maximum age was 75 years which depicts that there was no association between Rheumatoid arthritis and Gender (p-Value 0.71). The crosstab 1 showed the association (<0.001) between RA Factor and Anti-CCP as the 24 patients (80%) have positive anti-CCP while six patients (20%) found with normal Anti-CCP. Out of total 60 samples only 5 patients have positive ANA with 8.3% positive RA factor and 91.7% have negative RA factor while 83.3% normal patients have normal ANA results as shown in table 2. There is no association between Rheumatoid arthritis and ANA (p-value 0.0052). We performed TBARS assay on our samples with control group for oxidative stress. As per our results RA factor positive samples shows association (<0.001) with oxidative stress (TBARS). In Table 3 positive RA factor patients shows 70% significant correlation while negative patients showed 30% correlation with TBA. We Performed DPPH assay on our Patients samples as well as on control group but according to our results there was no association (p-value 0.492) between rheumatoid arthritis and DPPH as shown in figure 1. The frequency distribution of the statistical data was analyzed by Independent T test so the mean±SD of patient's age, Anti CCP, ANA, TBA and DPPH followed by 48.70±13.25, 292.93±346.03, 0.77±1.01, 23.71±8.44, 1.00±0.63 as well as of control group 46.27±13.89, 2.00±1.31, 0.15±0.09, 46.52±39.34, 0.21±0.52 respectively as shown in figure 2. In overall samples of rheumatoid arthritis Anti-CCP showed significant correlation at 0.001 with ANA and TBA, in patients' samples Anti-CCP showed significant correlation at 0.001 with TBA only, while in Normal group Anti-CCP and DPPH showed significant correlation at 0.05 (table 4). Paramod and associates, the current study included a cross sectional analysis. They examined 85 RA samples with the male and female ratio of 1:3.7 with mean age of 42.81±13.01 years. Study showed that Anti-CCP was positive even in RA patients who don't have positive RF results. This study shows that mostly RA factor was common in the age group of 40-50 years. [17] In order to do this, we examined 30 samples, 18 males and 12 female's patients ranging in age from 21-70 years then we compare with control group of 30 samples male (n=11), females (n=19) with the age of 15-70 years. Our study shows that 24 patients had positive RA factor and 6 patients did not show positive results for RA factor using Anti-CCP testing. The results of this study highlight the significance of Anti-CCP as a critical diagnostic marker in the evaluation of rheumatoid arthritis which may improve early identification and intervention techniques. As well as our study also concluded that rheumatoid arthritis is commonly in age group of 40-55 years.

Table 1: Sports infrastructure statistics for the Rabat-Salé-Kénitra Region [9]

	VolleyBall	HandBall	BasketBall	FootBall	Base nautique	Salle de Sport	Piscines	Stade de l'athlétisme	Stade de Rugby	Total
Rabat-Salé-Kenitra	24	26	33	53	3	21	12	13	5	190
Rabat	16	15	19	16	2	9	4	6	4	91
Kénitra	2	4	6	13	1	2	2	1	-	31
Khémisset	1	2	1	12	-	2	1	2	-	21
Salé-Aljadida et Salé-Médina	2	2	2	5	-	4	2	2	1	20
Sidi Kacem	1	2	3	4	-	2	2	2	-	16
Skhirate-Témara	2	1	2	3	-	2	1	-	-	11

Table 2: Gender distribution of the sample studied

Gender	**Percentage**
Male	70,2 b
Female	29,8 a
Total	100

Means in the same column with the same letter are not significantly different from each other at the 5% significance level.

Table 3: The Association of Anti- CCP with RA factor

Crosstab					
			Rheumatoid Arthritis Factor		Total
			Patients	Normal	
Anti CCP	Patients	Count	24	0	24
		% within Rheumatoid Arthritis Factor	80.0%	0.0%	40.0%
	Normal	Count	6	30	36
		% within Rheumatoid Arthritis Factor	20.0%	100.0%	60.0%
Total		Count	30	30	60
		% within Rheumatoid Arthritis Factor	100.0%	100.0%	100.0%

Table 4: Crosstab showed the results of RA factor and ANA

Crosstab					
			Rheumatoid Arthritis Factor		Total
			Patients	Normal	
ANA	Patients	Count	5	0	5
		% within Rheumatoid Arthritis Factor	16.7%	0.0%	8.3%
	Normal	Count	25	30	55
		% within Rheumatoid Arthritis Factor	83.3%	100.0%	91.7%
Total		Count	30	30	60
		% within Rheumatoid Arthritis Factor	100.0%	100.0%	100.0%

Fisher's Exact Test

Table 5: Cross-tabulation shows Association between RA and TBA.

Crosstab					
			Rheumatoid Arthritis Factor		Total
			Patients	Normal	
TBA	Patients	Count	30	12	42
		% within Rheumatoid Arthritis Factor	100.0%	40.0%	70.0%
	Normal	Count	0	18	18
		% within Rheumatoid Arthritis Factor	0.0%	60.0%	30.0%
Total		Count	30	30	60
		% within Rheumatoid Arthritis Factor	100.0%	100.0%	100.0%

Pearson Chi-Square 25.714a

Table 6: Spearman's correlation of Biochemical parameters

Rheumatoid Arthritis Factor		**A.N. A**	**DPPH**	**TBA**
Overall	Anti CCP	.702**	-0.031	.464**
	A.N. A		0.060	.443**
	DPPH			-0.121
Patients	Anti CCP	.471**	0.041	0.182
	A.N. A		-0.125	-0.011
	DPPH			.372*
Normal	Anti CCP	.402*	0.124	-0.216
	A.N. A		.448*	0.005
	DPPH			-0.356

Spearman's rho, *correlation significant at 0.05, **correlation significant at 0.001

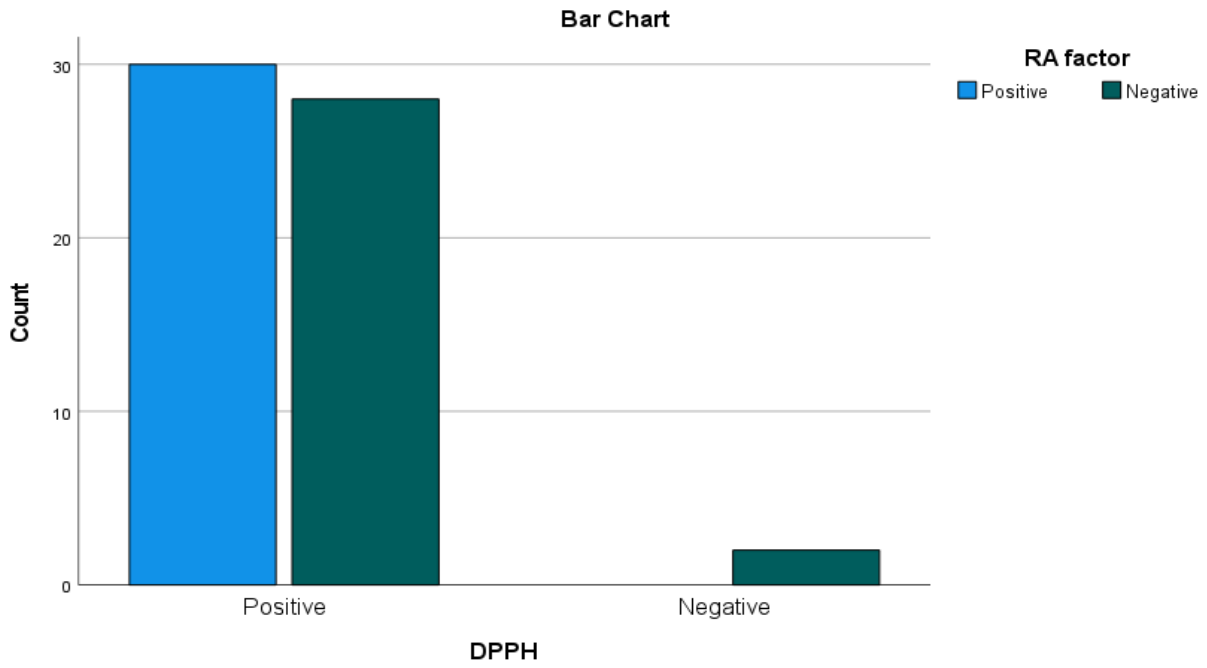

Figure 1. The graphical representation show that DPPH and RA has no association

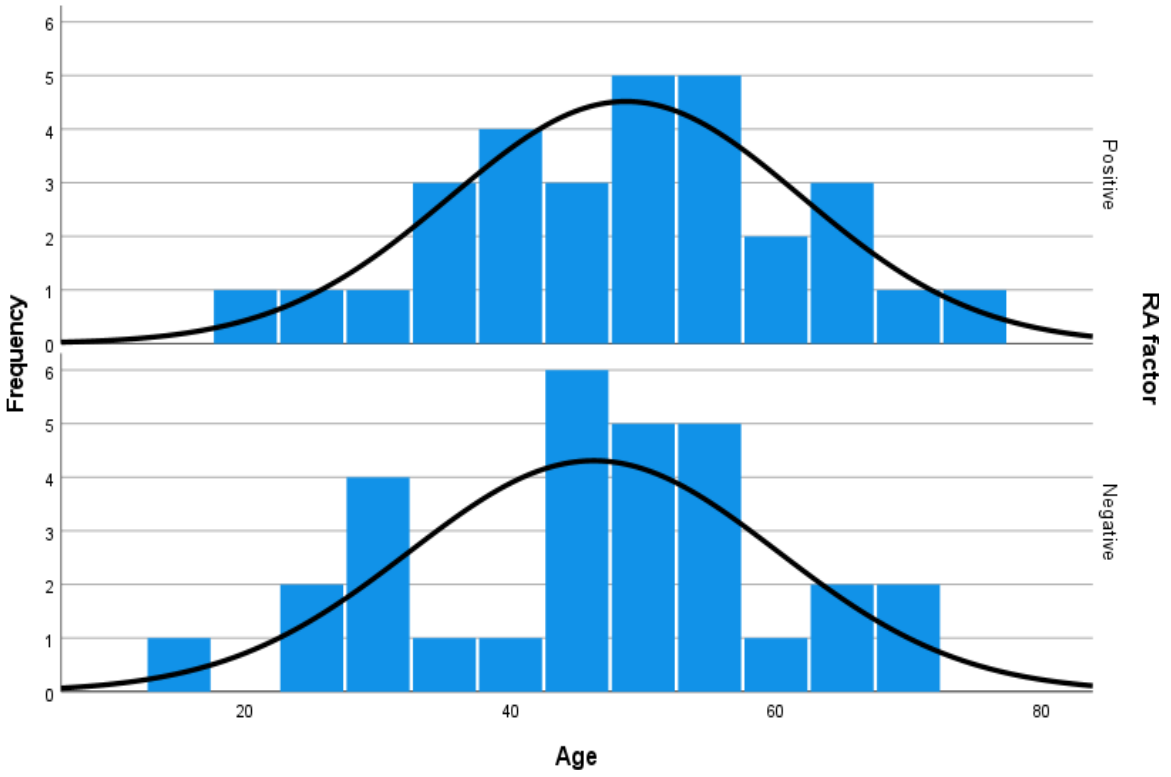

Figure 2: Showed the frequency distribution of RA factor with Age

Sujaytha and his colleagues described, 252 patients who had been diagnosed with Rheumatoid Arthritis (RA) and discovered that 161 of them (64%) had ANA testing only 25% had positive ANA tests. Interestingly, ANA-positive individuals were much younger and more likely to be CCP-positive than ANA-negative persons.[18] On the other hand, our research sought to explore the alleged lack of correlation between ANA and RA. In order to evaluate this, we used ANA testing to analyze 30 samples of RA patients, 12 female and 18 males, ranging in age from 21 to 70 years. We compared these RA patients to 30 normal subjects, 11 males and 19 females, ranging in age from 15 to 70 years. We found that 5 patients had positive RA factor results from ANA, while the other 25 did not. These findings were doubtful on the established relationship between ANA and RA.

Mirjana Veselinovic and colleagues conducted a study involving fifty-two Rheumatoid Arthritis (RA) patients (mean age 52.46 years, SD ± 7.39). Using the TBARS assay, they evaluated lipid peroxidation in plasma samples obtained from patients using sodium citrate anticoagulant. Their findings revealed significantly elevated TBARS levels in RA patients compared to healthy control subjects. Additionally, oxidative damage due to lipo-peroxidation products was observed in synovial fluids and tissues of RA patients. [19] In our study we used TBARS assay on rheumatoid arthritis patient's serum. We analyzed 30 samples from RA patients aged 21 to 70 years to evaluate their RA factor using TBARS assay. All RA patients displayed the presence of the RA factor through oxidative stress (TBARS) testing (100.0% within Rheumatoid Arthritis Factor). Our study showed significant correlation with TBARS assay. The findings from both studies collectively emphasize the association of elevated TBARS levels with RA, indicating its potential as a significant marker in assessing the oxidative stress characteristic of this autoimmune condition.[20] We performed antioxidative stress on RA factor positive samples by using DPPH method with biochemical parameters (ANA, Anti-CPP, and TBA). In our study DPPH did not showed any significant correlation with RA positive samples with p-value 0.492.

4. Conclusions

On the basis of our findings rheumatoid arthritis has significant association with Anti-cyclic citrullinated peptide and Thiobarbituric acid. According to our results oxidative stress may be causative agent of rheumatoid arthritis. So, it would be concluded that if anti-oxidative medicine is used as an administrative content for RA patients will be helpful for the treatment of RA patients as well as our researchers to do work on oxidative and anti-oxidative stress and also useful for pharmaceutical companies.

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