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The relationship between Serum Copper and some prognostic objective variables in patients with Rheumatoid Arthritis

ABSTRACT

Background: Rheumatoid arthritis (RA) is a progressive inflammatory disease, which could result in significant morbidity and mortality. Early and aggressive intervention with a new and effective biological treatment can alter the course of the disease, lengthen life, and improve function. Besides the rheumatoid factor (RF), another group of auto antibody has recently been detected in serum of patients with RA; the anti-cyclic citrullinated peptide antibodies (ACPA) which considered as a predictive of erosive disease with bad prognosis, in addition there are a number of prognostic variables that predict a poor outcome have been identified. Many studies found variable results regarding serum copper (S.Cu) levels in RA patients.

Objective: To assess the relationship between serum copper and some prognostic variables in rheumatoid arthritis.

Methodology: This study was a cross sectional study implemented in the Rzgary general teaching hospital in Erbil governorate-Kurdistan region/Iraq through the period of 12 months from 1st of November 2019, to 1st of November 2020. Study sample included fifty patients with RA. The diagnosis of RA was done according to 2010-American College of Rheumatology-European League against Rheumatism criteria. The S.Cu of patients was assessed in regard to variables of RA in the studied patients.

Results: There were a significant association between previous history of Cu wearing and high S.Cu level of RA patients (p=0.01), longer RA disease duration and high serum Cooper level (p<0.001), In addition, the positive ACPA, rheumatoid nodule and morning stiffness of patients were significantly related to high S.Cu level (p<0.001, p=0.001, p<0.001, respectively. There were a highly significant association between presence of bone erosions in x-ray of hand and high S.Cu level (p<0.001), anemia (p<0.001), high ESR level (p<0.001). While no significant differences were observed between S.Cu level regarding age (p=0.4), gender (p=0.1) and RF (p=0.75).

Conclusions: The serum copper level in rheumatoid arthritis patients was significantly related with many variables which considered as a predictor for disease prognosis. Encouraging physicians to adopt the serum copper level in assessment and monitoring of patients with rheumatoid arthritis.

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Introduction:

Rheumatoid arthritis (RA) is a progressive inflammatory disease, which could result in significant morbidity and mortality. It is one of the most frequent chronic inflammatory joint diseases as it affects approximately 0.5–1% of the world's population. It has a significant negative impact on quality of life, with job functioning as well as resultant health care costs to the community.

Early and aggressive intervention with a new and effective biological treatment can alter the course of the disease, lengthen life, and improve function (1).

Although the precise etiology of RA remains unknown, there is strong evidence for autoimmunity since several auto antibodies are associated with the disease. Besides the RF, another group of auto antibody has recently been detected in serum of patients with RA; the anti-cyclic citrullinated peptide antibodies (ACPA) (2).

Serological testing for (RF) has moderate sensitivity and specificity with some positivity in other chronic inflammatory and infectious diseases such as Sjögren's syndrome and chronic viral hepatitis prospectively (3).

The reported sensitivity and specificity of RF in current studies may be falsely elevated by the inclusion of RF as a diagnostic criterion in the commonly used American College of Rheumatology (ACR) diagnostic revised criteria for RA, which included IgM (RF) in addition to clinical and radiological criteria (4).

ACPA hold promise for earlier and more accurate diagnosis of disease, improved prognostic information, and have been implicated in RA pathogenesis. Accordingly, it has included in the new 2010 ACR/ EULAR criteria (5).

The first citrulline-binding auto antibodies in RA sera were discovered by Nienhuis *et al*. in 1964(6). The formation of antibodies to citrullinated peptides seems to be specific for RA patients (7).

ACPA has been reported to be highly specific (96%) and moderately sensitive

(78%) for RA and is seen in 70% of early RA. Furthermore, 30-40% of Seronegative RA is reported as ACPA positivity (8). In addition, ACPA positivity is predictive of erosive disease and bad prognosis (9).

There are a number of prognostic variables that predict a poor outcome have been identified and include female sex, strong family history, human leukocyte antigen-DR4 cluster susceptible genes, a high number of swollen/tender joints, high titer of RF, ACPA, elevated erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), and the detection of erosions on joint radiographs. In addition to a high score on Health Assessment Questionnaire (HAQ), low socioeconomic and educational status, presence of psychosocial problems and persistent pain (10).

The finding of abnormally high serum/plasma copper (CU) concentration in patients with RA has attracted interest. It has been claimed a correlation of S.Cu concentration with disease activity in RA (11). The most known changes in inflammation and infections are alterations in ferric ion (Fe), Zinc (Zn) and Copper (Cu) levels in sera associated with elevated levels of acute phase proteins (12). Many studies found variable results regarding S.Cu levels in RA patients (13).

This study aimed to determine the S. Cu and its association with group of variables well known to represent the prognostic factors of the diseases; like bone erosions, autoantibodies, rheumatoid nodules, morning stiffness and anemia, among patients with RA and its relations with age, sex, residence.

Materials and Methods

This is a cross-sectional study of 50 patients with RA, who were fulfilled the new 2010 ACR/ EULAR criteria for RA (5) and who attended to Rzgary General Teaching Hospital in the period of 12 months from 1st of November 2019-1st of November 2020 Erbil governorate in Iraq, which serves a population of more than one million.

The aim is to study serum level of Cu in RA patients and the association with other markers and autoantibodies known to be used as a prognostic factor of the disease, also to study its associations with age, sex, residence, among patients with RA.

Patients with conditions found to increase Cu levels-any other acute inflammatory conditions, renal, hepatic diseases, urinary tract infections, leprosy, malignancies, pregnant women, patients on oral contraceptive pills, patients currently wearing copper bracelets, ornaments or patients using copper utensils for cooking and feeding were excluded from the study.

Inform written consent was obtained from all patients, the approval of ethics committee was obtained from health ethics committee in hawleer medical university.

All patients undergo full medical history and physical examination. All patients were sent for complete blood count (CBC), erythrocyte sedimentation rate (ESR) was estimated by Westergren's method, C-reactive protein (CRP). ACPA was measured by enzyme-linked immunosorbent assay (ELISA) and RF by the agglutination method.

Fasting blood samples were collected for estimation of S.Cu level within 2 to 3 hours of blood-collection, in the department of biochemistry, by simple and sensitive colorimetric method (14).

X-rays of hands and wrist in postero-anterior (PA) views were taken for all patients, and its report written by an Experienced radiologist to detects any bone erosion.

The RA patients' information was interpreted statistically by SPSS program-26. Chi square and Fishers exact tests were implemented, and p value of ≤ 0.05 considered as significant.

Result

This study included fifty patients with. The mean age of patients was 50.04±9.7 years and range of (33-75 years) and predominant age category was ($\geq$ 50 years). Female gender was more than male gender with female:

male ratio (2.5:1). The residence of RA patients was urban for 52% while 48% of them are rural. Previous history of wearing copper were detected in 12% of them. More than half (58%) of studied patients had disease duration of ten years and more. Disease duration mean±SD (9.9±6.2 years) with range of (1-35 years). (*Table 1*)

Table 1. Basic demographic characteristics of the studied sample.

	No.	**%**
Age (years)		
< 30	**2**	**4%**
30-39	**8**	**16%**
40-49	**12**	**24%**
≥ 50	**28**	**56%**
Gender		
Female	**36**	**72%**
Male	**14**	**28%**
Residency		
Rural	**24**	**48%**
Urban	**26**	**56%**
wear or use CU		
Positive		
Negative	6	12%
	44	88%
Duration (years)		
< 1	**0**	**0%**
1-4	**11**	**22%**
5-9	**10**	**20%**
≥ 10	**29**	**58%**
Total	**50**	**100%**

Table 2. Variables considered as a prognostic factor of RA

Rheumatoid factor		
	Frequency	Percent
Positive	38	76%
Negative	12	24%
Total	50	100%
Anticitrullinated peptide antibody		
	Frequency	Percent
Positive	34	68%
Negative	16	32%
Total	50	100%
Rheumatoid nodule		
	Frequency	Percent
Positive	11	22%
Negative	39	78%
Total	50	100%
x-ray of hand PA for erosions		
	Frequency	Percent
Positive	27	54%
Negative	23	46%
Total	50	100%
Serum Copper ug/dl		
	Frequency	Percent
normal	23	46%
increase	27	54%
Total	50	100%
Hemoglobin gm/dl		
	Frequency	Percent
normal	21	42%
anemic	29	58%
Total	50	100%
Erythrocyte sedimentation rate		
	Frequency	Percent
normal	9	18%
mild	28	56%
moderate	5	10%
high	8	16%
Total	50	100%
morning stiffness		
	Frequency	Percent
Positive	32	64%
Negative	18	36%
Total	50	100%

Rheumatoid factor (RF) was positive in 76% patients, while the ACPA was positive among 68% patient l the rheumatoid nodule was positive among 22% patients. X-ray of hand with bone erosions was positive in 54% of RA patients. Mean serum copper level of RA patients was (147.2 mcg/dl); and 54% of RA patients had high S.Cu level.

Hemoglobin level of RA patients was normal in 42% while anemia found in 58% of them with hemoglobin level mean±SD (11.4±1.7 gm/dl). The Erythrocyte ESR level was normal in 18% of RA patients, while mild in 56% of them, moderate in 14% and high in the rest (12%), with mean±SD (44.4±27.5 mm/hr). Morning stiffness was positive in 64% of them. as showed in table 2.

No significant differences were observed between RA patients with normal S. Cu level and RA patients with high serum Copper level regarding age (p=0.4) and gender (p=0.1). ***(Table 3***)

Table 3: Distribution of demographic characteristics according to serum Copper level.

Variable	Serum Copper				P
	Normal		High		
	No.	%	No.	%	
Age					0.4*^{NS}
<40 years	6	26.1	4	14.8	
40-49 years	5	21.7	7	25.9	
50-59 years	7	30.4	13	48.1	
≥60 years	5	21.7	3	11.1	
Gender					0.1**^{NS}
Male	9	39.1	5	18.5	
Female	14	60.9	22	81.5	

**Fishers exact test, **Chi square test, NS=Not significant*.

No significant differences were observed between patients with normal serum Copper level and with high S.Cu level regarding residence (p=0.24). There was a significant association between previous history of copper wearing and high S.Culevel of RA patients (p=0.01). (***Table 4 and Figure 1***)

Table 4: Distribution of residence and Copper wearing history according to serum Copper level.

Variable	Serum Copper				P
	Normal		High		
	No.	%	No.	%	
Residence					0.24*^{NS}
Urban	14	60.9	12	44.4	
Rural	9	39.1	15	55.6	
Copper wearing history					**0.01****^{S}
Positive	0	-	6	22.2	
Negative	23	100.0	21	77.8	

**Chi-square test, **Fishers exact test, NS=Not significant, S=Significant* .

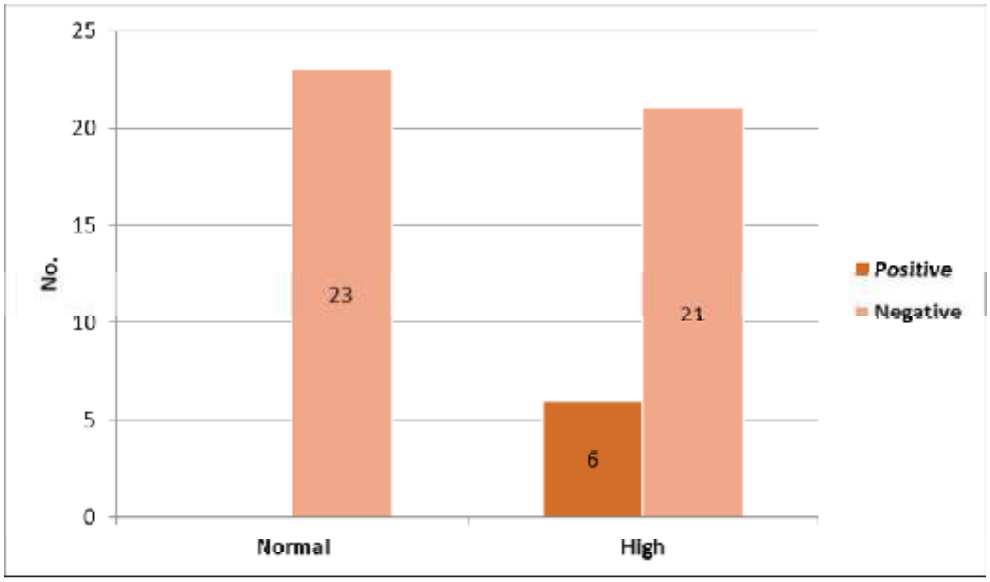

Figure 1: effect of history of copper wearing according to copper level. There was a highly significant association between longer RA disease duration and high S.Cu level ($p<0.001$). No significant differences were observed between patients with normal S.Cu level and high S.Cu level regarding RF ($p=0.75$). While the positive ACPA, rheumatoid nodule and morning stiffness of patients were significantly related to high S.Cu level ($p<0.001$, $p=0.001$, $p<0.001$, respectively). (***Table 5***)

Table 5: Distribution of RA prognostic variables according to serum Copper level.

Variable	**Serum Copper**				**P**
	Normal		**High**		
	No.	**%**	**No.**	**%**	
Disease duration					**<0.001***S
<10 years	19	82.6	2	7.4	
≥10 years	4	17.4	25	92.6	
Rheumatoid factor					0.75*NS
Positive	17	73.9	21	77.8	
Negative	6	26.1	6	22.2	
Anticetrulinated peptid antibody					**<0.001***S
Positive	7	30.4	27	100.0	
Negative	16	69.6	0	-	
Rheumatoid nodule					**0.001***S

Positive	0	-	11	40.7	
Negative	23	100.0	16	59.3	
Morning stiffness					**<0.001***S
Positive	5	21.7	27	100.0	
Negative	18	78.3	0	-	

**Chi-square test, NS=Not significant, S=Significant .*

There were a highly significant association between x-ray of hand with erosions and high serum Cooper level (p<0.001). The anemia in RA patients were significantly related to high serum Cooper level (p<0.001). A highly significant association was observed between high ESR level and high serum Cooper level (p<0.001). (***Table 6***)

Table 6: Distribution of other prognostic markers according to serum Copper level.

Variable	**Serum Copper**				**P**
	Normal		**High**		
	No.	**%**	**No.**	**%**	
X-ray of hand with erosions					**<0.001***S
Positive	0	-	27	100.0	
Negative	23	100.0	0	-	
Hemoglobin					**<0.001***S
Normal	21	91.3	0	-	
Anemic	2	8.7	27	100.0	
ESR					**<0.001****S
Normal	9	39.1	0	-	
Mild	14	60.9	14	51.9	
Moderate	0	-	7	25.9	
High	0	-	6	22.2	

**Chi-square test, **Fishers exact test, S=Significant .*

Discussion

The present study showed that mean age of RA patients was (50.04±9.7 years) and range of (33-75 years) with predominance of female gender. These findings are close to results of Al-Ani et

al (15) retrospective observational study in Iraq which reported that mean age of RA patients was (48.1 years) with predominance of female gender. Nilsson et al (16) study in Belgium found a significant difference in medical treatment and outcome in regard to RA patients' age and gender. The gender differences in RA diseases included differences in prevalence, disease progression, severity and treatment response. These differences might be attributed to many factors such as structural, hormonal and functional factors (17).

In current study, 52% of RA patients were urban residents, this finding coincides with results of Alkazzaz (18) study in Iraq which reported that 50.1% of RA patients were urban residents.

The Copper wearing history was observed in 12% of studied RA patients. This finding is similar to results of Richmond et al (19) study in UK which reported that some RA patients were wearing Copper Bracelets thinking that they had a therapeutic effect, but they did not have any effect.

Also, this study found that mean RA disease duration was (9.9years) and 58% of them had disease duration of ten years and more. This finding is close to results of Al-Rawi et al (20) study in Iraq on 111 patients with RA which revealed mean RA duration of (8.67 years) with predominance of patients with RA duration of more than ten years.

Our study showed RF in 76% of RA patients and ACPA in 68% of them. Bugatti et al (21) study in Italy reported the significance role of RF and ACPA autoantibodies in diagnosis and prognosis of RA in addition to monitoring and evaluation of treatment response and disease progression. Furthermore, in our study, the rheumatoid nodule was positive in 22% of RA patients and the morning stiffness was positive in 64% of them. These findings are close to results of Noori et al (22) study in Iraq which reported that rheumatoid nodule was recorded in 20% of RA patients and morning stiffness in 64% of them.

Current study showed that 54% of RA

patients had hands bone erosions in their x-rays, this finding is consistent with results of Attar and Al-Ghamdi (23) study in Saudi Arabia which revealed that radiographic changes in RA are helpful in diagnosis. Our study found that mean hemoglobin level of RA patients was (11.7 gm/dl) and 58% of them were anemic. Close to our findings, Ganna (24) study in Ukraine on 89 RA patients found that prevalence of anemia in RA patients were (64%).

The mean ESR of RA patients in our study was (44.4 mm/hr); 56% mild increase in ESR level, 14% moderate increase in ESR level and 12% high increase in ESR level. These findings are in agreement with results of Sokka and Pincus (25) study which revealed that majority of RA patients in USA and Finland had mild to moderate ESR increase between 1980 and 2004.

The present study found that mean S.Cu level of RA patients was (147.2 mg/dl); 54% of them had high level. These S.Cu results are close to outcomes of Xin et al (26) meta-analysis study in China that found high proportion of RA patients with high and S.Cu low serum zinc levels. The Copper (Cu) is a significant chemical constituent needed by normal human body for growing and developing. The Cu is required for maturating collagen tissues. Different literatures showed the Cu participation in pathogenesis of RA with various serum Cu levels among patients with RA (27-29).

Clearly in present study, there was a significant association between previous history of copper wearing and high serum Copper level of RA patients (p=0.01). This finding coincides with results of Li et al (30) study in Singapore which reported that long exposure to copper lead to elevation of serum S.Cu and hazardous effects. Historically, copper compounds have been used as paint pigments, wood preservatives and pesticides. Copper compounds are also used as actives or excipients in cosmetic and pharmaceutical products, e.g., copper peptide for skin regeneration purposes, cupric aspirinate and cupric salicylate

for RA treatment, copper conjugated dendrimer for anti- tumoral activity, and copper liposome for enhanced stability of doxorubicin. Copper can also be used in photo-thermal nanoparticles. These copper nanoparticles-mediated drug delivery systems can significantly increase the permeability of drug and enable controlled transdermal drug delivery (30).

The current study showed that longer RA disease duration, positive ACPA, rheumatoid nodule and morning stiffness of RA patients were significantly related to high S.Cu level ($p<0.05$). This finding is consistent with results of Strecker et al (28) study in Poland on 74 RA patients which documented that increased S.Cu level was detected in RA patients with activity and severity characteristics and signs.

This study revealed a highly significant association between each of positive x-ray of hands bone erosions, anemia and high ESR level with high S.Cu level ($p<0.001$). Consistently Youssef et al (31) study in UK found a significant relationship between high S.Cu level in RA patients and markers of RA activity like high ESR. A recent Polish study carried out by Tański et al (32) reported a direct link between high S.Cu level of RA patients and anemia.

Conclusions

-The serum copper level in rheumatoid arthritis patients was significantly related with many variables which considered as a predictor of disease prognosis.

Recommendations

The S.Cu level in rheumatoid arthritis patients was significantly related with many variables which considered as a predictor for disease prognosis. Encouraging physicians to adopt the S.Cu level in assessment and monitoring of patients with RA which might be useful in management plane of rheumatoid arthritis.

Further national longitudinal studies on role of S.Cu in diagnosis and staging of RA disease should be supported.

Study limitations

1. As other cross sectional studies,

the temporal relationship cannot be assessed.

2. Small sample size.

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