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## Levels of Zinc and Magnesium in Plasma of Sudanese Patients with Rheumatoid Arthritis

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#### ABSTRACT

Zinc (Zn) and magnesium (Mg) have many important functions in human body. Among many contributing agents that have been proposed to take part in the pathogenesis of rheumatoid arthritis RA condition, trace elements, including Zn and Mg have also been investigated. This study aimed to evaluate the level of these elements and their correlations with duration and complications of RA. the plasma level of trace elements was estimated by the use of Atomic Absorption Spectroscopy. This cross-sectional hospital base study involved 45 rheumatoid arthritis patients compared with normal reference values of the trace elements. The analysis of the results indicated that the mean plasma levels of Zn and Mg were significantly lower when compared with their reference values, found lower levels below the lower limit of the reference value, reveals a statistical significant difference P.value (0.000, 0.000). Correlations between plasma Zn, Mg levels and complications of disease were found insignificant whereas positive correlations between the level of Zn and duration of RA disease, r=(0.4), p. value was (0.005) and between Mg level and Zn r=0.3, p. value was (0.04)patients with RA have lower levels of Zn and Mg, so the supplementation of Zn and Mg would be necessary for RA patients to correct their levels.

Keywords: RA patients, Trace elements

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#### INTRODUCTION

Rheumatoid arthritis (RA) is an immune-mediated chronic inflammatory disease. Despite being characterized by inflammation of the synovial membrane and progressive destruction of the articular cartilage and bone. RA is a systemic disease often associated with other extraarticular manifestations, with a significant impact on mortality and morbidity<sup>1</sup>.

Many of symptoms are present before its diagnosis. Weight loss, fever, prolonged early morning stiffness, fatigue, generalized muscle weakness, low mood, and depression are often responsible for a significant loss in the quality of life of patients. Fatigue is reported in 40–80% of RA patients as their most disabling symptom <sup>2</sup>. Among many contributing agents that have been proposed to take part in the pathogenesis of this condition, trace elements, including Zn have also been investigated <sup>3</sup>.

Trace elements are widely distributed in a variable proportion in human body and they play a vital role in growth <sup>4</sup>. Zinc (Zn) is an important for the proper functioning of more than 300 hormones and enzymes in the human body <sup>5</sup> that bound to proteins, predominantly albumin,  $\alpha$ 2-macroglobulin and transferrin. Zinc is transported to the cells but only free zinc ionsseem to be biologically active<sup>6,7</sup>. Although the intriguing role of zinc as an essential trace element for immune function has already been well established<sup>8,9</sup>. The reduction of zinc concentrations can result in growth retardation<sup>10,11</sup> decreased natural killer activity, and altered cytokine production <sup>10</sup>.

T-cell function is impaired by reduced zinc availability leading to reduced T-cell numbers <sup>12</sup>.Zinc is an essential cofactor for thymulin, an important thymic hormone secreted by thymic epithelial cells <sup>13, 14</sup> and thymus changes are reversible by zinc substitution<sup>15</sup>. Thymulin induces markers of differentiation in immature T cells in the thymus but also has effects in the periphery <sup>16</sup>. Zinc also influences mature T cells and it induces the expression of the highaffinity receptor for IL-2<sup>17</sup> and zinc deficiency is associated with decreased T cell proliferation after mitogen stimulation <sup>18,19</sup> furthermore Zinc has a significant role in antioxidant processes because it is a constituent of superoxide dismutase(SOD)<sup>20</sup>. Zinc is responsible for the functions of metallo proteinases through abond. The breaking of this bond and release of the pro-peptide can lead to the activation of metallo proteinases that are responsible for articular cartilage destruction in RA<sup>21</sup>. In previous studies it was found that zinc level decreases in RA patients <sup>22,23</sup>. Magnesium (Mg) is a cofactor involved in many enzymatic systems it being necessary for protein synthesis, functioning of nervous and muscular systems, regulation of blood pressure and glycaemia, bone metabolism. It also play a role in the active transport of calcium and potassium ions across cell membranes, and through these processes influences nerve conduction, muscular contraction and cardiac

rhythm<sup>24, 25</sup>. Arole of magnesium is achieved through two important properties the ability to form chelates with important intracellular anionic-ligands, especially ATP, and its ability to compete with calcium for binding sites on proteins and membranes <sup>26</sup>. This synchronization whereby magnesium induces muscle relaxation and calcium aids muscle relaxation brings about movement. Magnesium deficiency may lead to cardiovascular disease and immune dysfunction, which h its adequate presence ought to protect <sup>27</sup>. Its presence is necessary for both aerobic(oxidative phosphorylation) and anaerobic(glycolysis) energy production processes, both indirectly, as a component of ATP-Mg complex and directly, as an enzymatic activator<sup>28</sup>. It is essential in osteoblasts and osteoclasts functioning, vitamin D activation, calcitonin release and suppression of parathyroid hormonerelease<sup>29</sup>. Deficiency of Magnesium can produce a syndrome of vitamin D resistance<sup>30</sup>. Vitamin D enhancing chemotaxis and phagocytic capabilities of innate immune cells <sup>31</sup>, while active form of vitamin D severely affects T cell activation, proliferation and differentiation, it facilitates the production of regulatory T (Treg) cells that can indeed function as an effective immune controller<sup>32, 33</sup>. Magnesium supplement has a great effect on depression and muscles function as well as the smooth muscles of blood vessels and the gastrointestinal tract <sup>34</sup>. Some fuels cannot be stored in the muscle cells unless adequate supplies of magnesium are available, the metabolic role of magnesium is so diverse that it is difficult to find a body system that is not affected by magnesium deficiency<sup>35</sup>. In previous studies it was found that magnesium level decreases in RA patients <sup>36, 37</sup>.

### MATERIALS AND METHOD

#### Study design:

Descriptive Cross sectional study involved 45 RA patients with both gender. All patients were selected through direct questionnaire from the Military Hospital and Omdurman Hospital, part of Khartoum state. The study period spanned from (October to December, 2015). The aim of the study was to evaluate the association between plasma levels of Mg, Zinc and the incidence of RA patients in Sudanese as well as their correlations.

#### **Patients and Method:**

This study involved 45 patients with RA (5 males and 40 females their ages ranged between 18-75 years. The duration of disease was (0.5-25 years), mean (6.83) years. Blood samples were collected from patients to assess serum levels of zinc and magnesium, that was compared with reference value of Zn (0.5 - 1.2mg/l) and Mg (17 - 28mg/l). (Table 1) describes the distribution pattern of the quantitative parameters in RA patients. Blood samples were collected from all patients in lithium heparin containers. After verbal consent from all participants, blood samples were centrifuged for 10 minutes at 3000-4000 rpm, and

the plasma was separated in new containers and well-kept, stored at -20c<sup>o</sup> till tested for Mg and Zinc levels. Atomic Absorption Spectrophotometry was used for determination of plasma Mg, Zinc. The analysis was done at the Environmental, Natural Resources and Desertification Research Institute in Khartoum city.

The protocol of the study was approved by the Ethical Committee of College of Medical Laboratory Sciences, Elneelin University. Verbal consent was obtained from all subjects before participation. All patients had fulfilled the American College of Rheumatology (ACR) criteria<sup>38</sup>.

Inclusion criteria: Only diagnosed RA patients by physician rheumatologist

**Exclusion criteria:** Any Pregnant patients or had recent infection was excluded from this study.

#### **Statistical Analysis:**

SPSS software was used for statistical analysis. T-test was used to find association between the level of trace elements compared to reference value and ANOVAwas used to find correlation between duration and complication of RA with level of trace elements9Zn, Mg). Statistical significance was defined as p < 0.05.

#### **RESULTS AND DISCUSSION**

In this study mean plasma levels of Zn and Mg were significantly lower when compared with normal reference value with p.value (0.00, 0.00), respectively. Our result findings showed that plasma Zn level was decreased in almost all patients, meanwhile the Mg was found low in about 36 (77.7%) of the patients.

Table 2 described the duration of disease correlated with level of trace element (Zn, Mg), the duration was divided in to 3 groups, first one less than 5 years, second from 5 to 10 years and the third one more than 10 years. The higher mean level of Zn was found in the  $3^{rd}$  duration group, and was positively correlated with duration of the disease, p.value (0.000). Mean levels of magnesium and age in the three duration groups were statistically insignificant, p.value (0.19, 0.52).

Table 3 described the complication of disease correlated with the level of trace elements (Zn, Mg). The complications encompasses 3 groups according to severity of disease. Group1 have slight complication:(pain in joint, morning stiffens, fatigue, fever).Group 2 has moderate complication (swelling in joint of hand or foot or knee, shoulder tenderness). Group 3 have severe complication: (deformation of joints or affect other organ).

Figure (1) presents correlation between the level of Zn and duration of RA disease, r=0.4, p.value=0.005, whereas between Mg level and Zn r=0.3, p.value=0.04 figure (2).

Table 1: shows study group characteristics of (RA patients, no = 45)

	Range	Median	Mean±SD	p.value
Duration of disease/years	0.5-25	5.0	6.8±5.9	
Zn (mg/l)	0.054-0.34	0.15	$0.16 \pm 0.07$	0.00
Mg (mg/l)	5.65-24.95	13.5	14.1±4.1	0.00

Table 2: Presents correlation between age and level of zinc and magnesium with

A disease	RA	of	ation	lur

	<5	5-10	>10	p.value
Mean Zn level	0.14±0.039	$0.14 \pm 0.07$	$0.28 \pm 0.03$	0.000
Mean Mg level	14.3±4.9	13.4±3.0	$17.0\pm 5.4$	0.192
Mean Age group	43.9±12.7	46.8±12.6	$50.8 \pm 5.8$	0.521

 Table 3: Correlation between the mean level of trace elements and complications of RA

 disease

Trace elements	Slight	Moderate	Sever	Normal range
	complication	Complication		in (Mg/l)
Zn Mean ±SD	$0.18 \pm .058$	$0.15 \pm .074$	$0.16 \pm .073$	0.5-1.2
Mg Mean ±SD	15.7±3.6	14.2±4.2	13.0±4.0	17-28



Figure:1:shows correlation between Zn level and duration of disease among RA patients.





This study aimed to evaluate the levels of trace elements (Zn, Mg) in RA patients as well as to study correlations between these with complications and durations of RA disease. The role of trace elements in chronic inflammatory states is of great interest because many of them are co-factors in metabolic processes involving articular tissues and immune system function <sup>40</sup>. In Table (1) effect of RA disease in the level of Zn and Mg, in this study plasma Zn level was severely decreased in almost of all patients, and was significantly different when compared with lower limit of normal reference value p.value (0.000), the result of this study was in agreement with previous studies<sup>22</sup>, whose study results indicated significantly lower levels of Fe, Cu, and Zn in the biological samples (blood and scalp hair) as compared to control subjects of (p < 0.01).Moreover In other research study <sup>23</sup> the results showed that the concentration of Zn was lower in the biological samples of rheumatoid arthritis patients of both genders with respect to non-arthritic subjects.

The significant reduction of Zn level was due to its malabsorption in RA patients. Previous study stated that when zinc was given to healthy volunteers and patients with RA, it was found that the level has increased in the healthy group but the level has not changed in the patients with RA disease<sup>40</sup>. Or may be due to its high utilization because it is an antioxidant and as a constituent of the antioxidant enzyme structure SOD<sup>20</sup>, that was found significantly increased in erythrocyte of RA patients<sup>41</sup>. In this study the plasma level of Mg element was found low in about 36 (77.7%) of the patients, and showed statistical significant difference in our RA patients p.value (0.000) in comparison with lower normal limit value. This results of our study agreed with two previous, first <sup>36</sup>, whose study results indicated significantly lower levels of Ca, Mg, and K in the biological samples (blood, serum, and scalp hair) of male and female rheumatoid arthritis patients when compared to referents of both genders, and the second study<sup>37</sup> was Evaluated the serum magnesium and lipid profile, various biochemical parameters as risk factors of cardiovascular diseases in patients with rheumatoid arthritis and it was found decreased serum magnesium and calcium in RA subjects compared to the controls. Correlations between level of (Zinc, Magnesium)and duration of disease in RA patients, when analyzed revealed that Zinc was positively correlated with the duration of disease, p.value (0.000) but magnesium and age was insignificant (table 2).Correlations study between level of Zinc, Magnesium and complications of RA disease, was not significantly different (Table 3). Correlation was found positive between the level of Zn and duration of RA disease, r=0.4, p.value=0.005, whereas between Mg level and Zn r=0.3, p.value=0.04 (figure1, 2).

### CONCLUSION AND RECOMMENDATION:

The results observed that the levels of plasma Zinc and magnesium were significantly

decreased in patients with Rheumatoid arthritis as compared to reference value. Insignificant correlation observed between level of this element and complications of RA. A significantly low level of Zinc with duration of disease was observed but Magnesium not significant.

A Supplementation with zinc and magnesium would be necessary for RA patients, because low zinc and magnesium have a significant role in pathogenesis in RA patient, and even more important to individuals who are at high risk of developing RA. This study presents guidance to clinicians and other professional investigating deficiency of Zinc and Magnesium in RA patients. Prospective study with large sample size should be conducted for further investigations of RA patients.

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