Scholars Journal of Applied Medical Sciences
Abbreviated Key Title: Sch J App Med Sci
ISSN 2347-954X (Print) | ISSN 2320-6691 (Online)
Journal homepage: www.saspublishers.com

OPEN ACCESS

Biochemistry

Serum Zinc level in Rheumatoid Arthritis in a Tertiary Care Hospital, Dhaka, Bangladesh

Dr. Md. Ruhul Quddus[1*], Dr. Syed Golam Gausal Azam Quadri[2], Dr. Md. Jahangir Alam[3], Dr. Md. Ismail Hossain[4]

[1]Assistant Professor, Department of Biochemistry, Pabna Medical College, Pabna, Bangladesh
[2]Senior consultant (Chest disease), OSD, DGHS, Attached with 250 bedded specialized General Hospital, Jashore, Bangladesh
[3]Senior Consultant (ENT), Adhunik Sadar Hospital, Nilphamari, Bangladesh
[4]Senior consultant (Dermatology), 250 bedded general Hospital, Jamalpur, Bangladesh

DOI: 10.36347/sjams.2019.v07i08.009 | **Received:** 02.07.2019 | **Accepted:** 25.07.2019 | **Published:** 18.08.2019

***Corresponding author:** Dr. Md. Ruhul Quddus

Abstract | **Original Research Article**

Background: Rheumatoid arthritis is a chronic inflammatory, auto-immune disease characterized by inflammatory synovitis involving symmetrical peripheral joints with increased risk of cardio-vascular events and osteoporosis. Zinc acts as a co-factor of important enzymes involved in collagen and bone metabolism, the antioxidant defense system and the immune system. Serum zinc level is altered in the patients with rheumatoid arthritis. The purpose of this study was to assess the relationship of serum zinc level in the patients with rheumatoid arthritis. ***Methods:*** This cross-sectional analytical study was conducted on 50 patients of rheumatoid arthritis and 50 healthy individuals with age, sex and BMI matched in the department of Biochemistry, Dhaka Medical College, Dhaka from July 2014 to June 2015. Patients suffering from DM, CRF, malignancy, any infection, respiratory diseases, liver diseases or recent history of diarrhea and dysentery were excluded. Serum zinc level of all the study subjects were measured by colorimetric method in the department of Biochemistry, Dhaka Medical College, Dhaka and compared statistically. For statistical analysis Student's t-test, Chi-square test and Pearson's correlation test was performed using SPSS for windows version 16. ***Results:*** Most of the patients were in 3rd to 4th decade in both groups. Mean ±SD age of group A and group B were 38.80±10.15 and 35.32±6.59 respectively. The mean ±SD BMI of group A and group B were 24.46±1.61 and 25.47±3.96 respectively. Among the study subjects the male female ratio was in group A- 1:2.57 (M-14, F-36) and group B- 1:2.33 (M-15, F-35). Level of education, occupational status and socio- economic status were almost alike between two groups. The mean ±SD of serum zinc level were significantly lower in group A (56.14±8.64) than group B (90.38±14.03), p<0.001. The mean ±SD of ESR were significantly higher in group A (79.94±12.21) than group B (9.92±1.99), p<0.001.A strong negative correlation (r) was found between serum anti-CCPA and serum zinc (anti-CCPA, r= -0.704, p<0.001) which was highly significant statistically. A negative correlation (r) was found between inflammatory markers serum C-reactive protein (CRP) and erythrocyte sedimentation rates (ESR) with serum Zn (CRP, r= -0.613 p<0.001 and ESR, r= -0.544, p<0.001) which was significant statistically. A weak negative correlation (r) was found between serum RF with serum zinc (RF, r= -0.413, p=0.003) which was also significant statistically. ***Conclusion:*** Hypozincemia is associated with rheumatoid arthritis. As rheumatoid arthritis is an autoimmune inflammatory disorder and zinc acts as a important co-factor for the enzymes which are involved in the normal inflammatory process and proper functioning of immune system, routine check-up regarding zinc status will help to prevent co-morbidities associated with the disease. Routine monitoring of serum zinc level will also help to limit the long term health hazards of rheumatoid arthritis.

Keywords: Serum Zinc, level, Rheumatoid Arthritis.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterized by inflammation of joints those results in cartilage and bone destruction, joint deformity and loss of mobility. Symmetrical inflammatory poly-arthritis is the primary clinical manifestation. The small joints of hands and feet are mainly affected [1]. The precise etiology of RA remains unknown, but a variety of inherited, hormonal and/or environmental factors may be important.

Rheumatoid arthritis has a prevalence of about 1-2% worldwide. In The United States, the prevalence

is ranged from 2.03%-2.72%; in Europe, 0.33% to 0.92% and in Asia-Pacific region 0.12% to 0.75% [2]. The onset is most frequent during the 4th& 5th decades of life with 80% of all the patients developing the disease between the ages of 35-50 years [3]. Rheumatoid arthritis is 2-3 times more frequent in women than men [4].

In Bangladesh, rheumatoid arthritis is a common inflammatory articular disorder and the prevalence and incidence is 0.2% to 0.7% and 120 per 100 000 / person years respectively [5]. According to WHO-ILAR COPCORD study 2006, RA was 4.09% and 36.06% among musculoskeletal disorders and inflammatory arthritis in Bangladesh respectively and females were more common (ratio 4.5:1).RA is diagnosed clinically, but classified according to the 2010 American College of Rheumatology (ACR) and European League Against Rheumatism (EULAR) classification criteria. Anti-CCPA and anti-MCV assay, are common serological tests, for early diagnosis of RA with specificity around 95%. Recently, point-of-care test (POCT) has been developed for early detection of RA and shows a sensitivity of 72% and specificity of 99.7%. Others Investigations include CBC, ESR, Rheumatoid factor (RF), C-reactive protein (CRP) and X-ray of involved joints. Zinc is a crucial element in a series of cellular functions as normal growth, protein metabolism, membrane stability, and metalloenzyme functions. It is a component of more than 300 enzymes from all six enzyme classes, serves as structural ions in transcription factors and is stored and transferred in metallothioneins [6]. About 10% of the human proteome (2800) contain zinc binding motives [7]. As cofactor of several enzymes associated with collagen and bone metabolism and immune function, role of zinc is important in chronic inflammatory disease. Maret W *et al.*, [6] suggested that, increased zinc deficiency might be involved in the development and progression of rheumatoid arthritis. According to Jelly and others in 2012, 38.34% had hypozincemia among female rheumatoid arthritis patients in Bangladesh. Serum zinc level is significantly reduced in patients with rheumatoid arthritis, potentially due to malabsorption [8]. It was reported that inflammation in rheumatoid arthritis causes decrease serum level of zinc resulting from zinc sequestration in the liver, pancreas and intestine due to cytokine induced metalloenzyme synthesis. Moreover, decrease serum zinc level results in T- lymphocyte function disorder which is involved in the pathogenesis of rheumatoid arthritis [9]. Zinc deficiency may lead to secretion of pro-inflammatory cytokines [10] and altered imbalance of cell-mediated and antibody-mediated immunity [11]. There is great socio-economic impact of rheumatoid arthritis in the society. RA was estimated to be the 40thleading cause of non-fatal burden in the world in 1990 [12] and 42nd highest contributor to global disability [13]. Around 40% of RA patients are disabled within 3 years; around 80% are moderately to severely disable within 20 years; and 25% will require a large joint replacement [14]. Huge number of lost working days and massive reduction of the work output owing to increased mortality and morbidity, approaching 3% of GDP [15]. The associated complications of osteoporosis and cardio-vascular events make RA important in public health terms due to elevated production of the inflammatory cytokines IL-1b and TNF-α. A negative correlation between the serum level of zinc and the levels of the pro-inflammatory cytokines IL-1b and TNF-α was found [8]. Zinc is found in alpha macroglobulin, an important protein in the body's immune system and affected by changing zinc levels, due to its high cell turnover [16]. Zinc is part of some important antioxidant compounds, including superoxide dismutase (SOD) and zinc monomethionine and protects the body from chemical damage, helping with detoxification of the body. Rheumatoid arthritis is a progressive auto-immune disease [17]. Conventional treatments are rarely, totally effective and causes potential side-effects, like gastro-intestinal bleeding and bone loss [18]. Some drug therapies have anti-nutrient effects by both increasing the requirement of some nutrients and reducing their absorption. Studies have suggested that, diet may play a role in the management of RA, particularly in alleviating the symptoms of the disease and reducing the risk of complications [19]. Maret and Sandstead [6] found that zinc significantly reduced joint swelling, morning stiffness, and improved patient's subjective assessment of disease activity. Large numbers of subjects in Bangladesh are affected by rheumatoid arthritis. Females are more affected than male. Low socio-economic status of Bangladeshi people has an increased risk of zinc deficiency [9]. With this background this study has been undertaken to observe the serum zinc levels in rheumatoid arthritis patients in our country to find out the possible role of zinc in rheumatoid arthritis. Therefore, keeping all such important points and views in mind, the focus and aim of this study is to evaluate and assess the nutritional status of zinc in patients with rheumatoid arthritis. So far, no recent published data available of zinc status in the patients with RA in Bangladesh. As such this study will fill the gap, open a new forum of discussion and will provide knowledge and information regarding the medical workup of patients with rheumatoid arthritis as well as to reduce the complication.

OBJECTIVES

General Objective

- To observe serum zinc level in the patients with rheumatoid arthritis.

Specific Objectives

- To estimate serum zinc level in the patients with rheumatoid arthritis.
- To estimate serum zinc level in healthy controls.
- To compare the serum zinc level between cases and controls.
- To estimate serum anti-CCPA, serum RF, CRP, ESR in RA patients.

MATERIAL AND METHODS

The present study was a cross sectional comparative study, which was conducted in the Department of Biochemistry, Dhaka Medical College, Dhaka during the period from July, 2014 to June, 2015. A total number of 100 subjects of both sexes were selected purposively according to selection criteria with age ranging from 21 to 65 years. Among them, 50 subjects were rheumatoid arthritis patient (Group A-case) and 50 were healthy individual (Group B-control). Case was selected from Rheumatology clinic in Physical Medicine Department and Medicine ward of Dhaka Medical College Hospital, Dhaka. After selection , the objectives, nature, purpose and benefits of the study were explained in detail in each study subject and informed written consent was taken. Data were collected in pre designed data collection sheet with a structured questionnaire formed by the researcher himself. Age, sex, occupation, marital status was noted as per statement of the participants at the time of interview. History regarding smoking habit, hypertension, diabetes mellitus, pregnancy, history of any cardiovascular event, thyroid disorder, chronic liver disease, any malignancy was taken. Drug history regarding nutritional supplement including zinc was noted. Then participants were examined for height, weight, blood pressure. The study parameter was serum zinc level. Estimation of study parameter was done in the Department of Biochemistry, Dhaka Medical College, Dhaka. Serum zinc level was assayed on semi-automated biochemical analyzer.

RESULTS

A cross sectional analytical study was conducted in the Department of Biochemistry, Dhaka Medical College, Dhaka during the period from July, 2014 to June, 2015. A total 100 subjects were selected. Among them, 50 subjects were in Group - A (case-rheumatoid arthritis) and 50 subjects were in Group - B (control-healthy individual). All the study subjects were selected purposively according to the selection criteria. Serum zinc and ESR level were assessed in both groups and compared to observe the association among the groups. Serum anti-CCPA, serum RF and serum CRP were done in the patients with rheumatoid arthritis and observed the correlation with serum zinc. The results and observations obtained were presented below.

Table-1: Demographic characteristics of the study subjects (n=100)

Demographic characteristics	Group A (n=50) No. (%) Mean ±SD	Group B (n=50) No. (%) Mean ±SD	p value
Age (years)	38.80±10.15	35.32±6.59	0.05^{ns}
Weight (Kg)	64.72±5.17	65.52±9.54	0.60^{ns}
Height (m)	1.61±0.09	1.63±0.06	0.15^{ns}
BMI (Kg/m^2)	24.46±1.61	25.47±3.96	0.09^{ns}

Table-1 shows the demographic characteristics of the study subjects. The results were expressed in mean ±SD. The age distribution was almost similar in group A and group B which were 38.80±10.15 and 35.32±6.59 respectively, p=0.05 which was not statistically significant. BMI in group A and group B was 24.46±1.61 and 25.47±3.96 respectively, p=0.09 which was also not statistically significant.

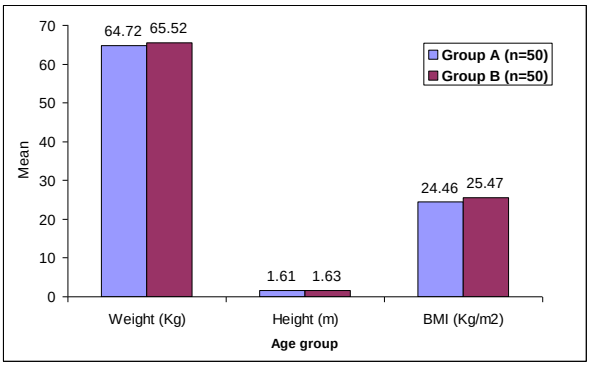

Fig-1: Bar diagram shows demographic distribution of the study subjects

Table-2: Distribution of the study subjects by sex (n=100)

Sex	Group A (n=50) No. (%)	Group B (n=50) No. (%)	χ^2	p value
Male	14(28.0%)	15(30.0%)	0.05	0.825^{ns}
Female	36(72.0%)	35(70.0%)		
Total	50(100.0%)	50(100.0%)		
M:F ratio	1:2.57	1:2.33		

Table-2 shows the sex distribution of the study subjects. It was observed that rheumatoid arthritis was more common in female than male. There were 14(28.0%) male, 36(72.0%) female in group A and 15(30.0%) male, 35(70.0%) female in group B, p=0.825 which were not significant statistically. Male female ratio was 1:2.57 and 1:2.33 in group A and group B respectively.

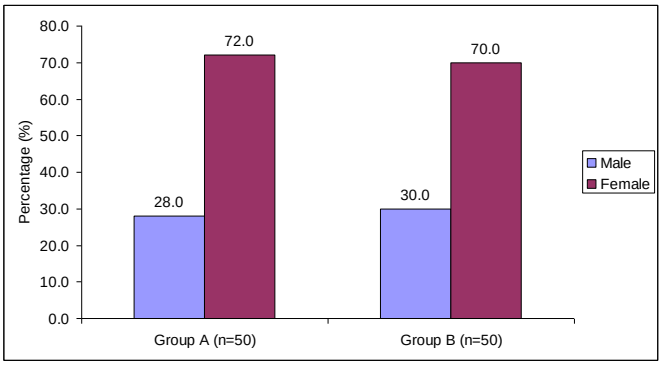

Fig-2: Bar diagram shows sex distribution of the study subjects (n=100)

Table-3: Distribution of the study subjects by occupation (n=100)

Occupation	Group A (n=50) No. (%)	Group B (n=50) No. (%)	χ^2	p value
Service	5(10.0%)	13(26.0%)	5.019	0.170^{ns}
Business	8(16.0%)	7(14.0%)		
Household worker	34(68.0%)	29(58.0%)		
Others	3(6.0%)	1(2.0%)		
Total	50(100.0%)	50(100.0%)		

Table-3 shows the occupational distribution of the study subjects. It was observed that almost two third 34(68.0%) of the patients in group A were involved in the household works, not worked in outside and 29(58.0%) were in control group, p=0.170. The difference was not significant statistically regarding occupational status between two groups.

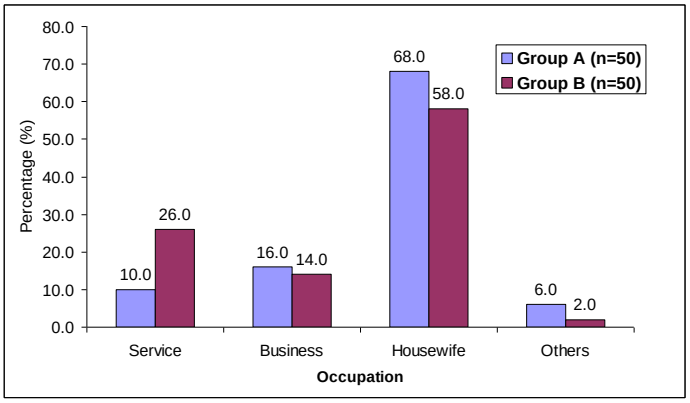

Fig-3: Bar diagram shows the occupational status of the study subjects (n=100)

Table-4: Distribution of the study subjects by socioeconomic status (n=100)

Socioeconomic status	Group A (n=50) No. (%)	Group B (n=50) No. (%)	χ^2	p value
Lower	42(84.0%)	36(72.0%)	2.098	0.148^{ns}
Middle	8(16.0%)	14(28.0%)		
Total	50(100.0%)	50(100.0%)		

Table-4 shows the socio-economic status among the study subjects. It was observed that rheumatoid arthritis is more common in poor class people, 42(84.0%) than in middle class, 8(16.0%), p=0.148 which was not significant statistically.

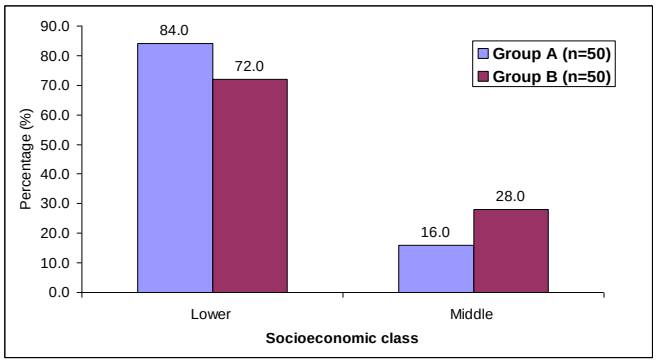

Fig-4: Bar diagram shows the socio-economic status of the study subjects

Table-5: Laboratory investigations in two study groups (n=100)

Laboratory investigations	Group A (n=50) No. (%) Mean ±SD	Group B (n=50) No. (%) Mean ±SD	t value	p value
Serum zinc	56.14±8.64	90.38±14.03	-14.69	<0.001*
ESR	79.94±12.21	9.92±1.99	40.02	<0.001*

Table-5 shows the laboratory investigations of the study subjects. The results were expressed in mean± SD. It was observed that serum zinc level was lower in group A (56.14±8.64) than group B (90.38±14.03), p=0.001 which was highly significant statistically. It was also observed that Erythrocyte Sedimentation Rate (ESR) was much higher in group A (79.94±12.21) than group B (9.92±1.99), p=0.001 which was statistically significant (p<0.05).

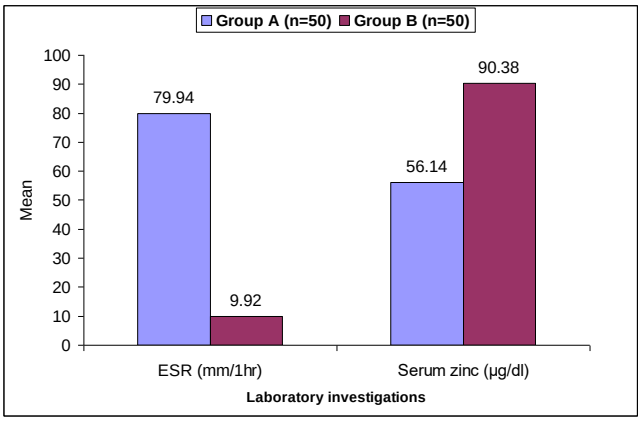

Fig-5: Bar diagram shows the status of the laboratory investigation of study subjects

Table-6: Correlation of serum zinc with anti-CCPA, CRP titer, and ESR in the study group (n=50)

Parameters	Group A (n=50)	
	r	p
Anti-CCPA	-.704**	<0.001
CRP titer	-.613**	<0.001
ESR	-.544**	<0.001
RF	-.413**	0.003

**** Pearson's Correlation Coefficient test, Pearson's Correlation coefficient (r) test was performed to compare relationship between serum zinc and anti-CCPA, CRP titer, ESR, RF. The test of significance was calculated and p value <0.05 was accepted as level of significance.**

Table-6 shows the correlation (r) between serum zinc and serum anti-CCPA, serum CRP, serum ESR and serum RF. It was observed that serum zinc had significant negative correlation with serum anti-CCPA (r= -0.704, p=0.001), serum CRP (r= -0.613, p=0.001), serum ESR (r= -0.544, p=0.001) and serum RF (r= -0.413, p=0.003).

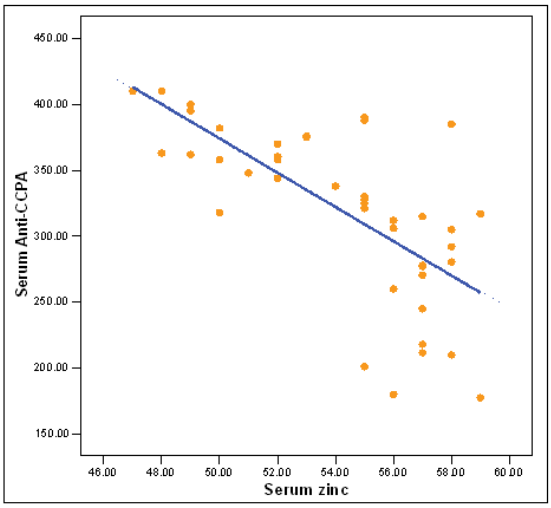

Fig-6: Correlation of serum zinc with serum anti-CCPA

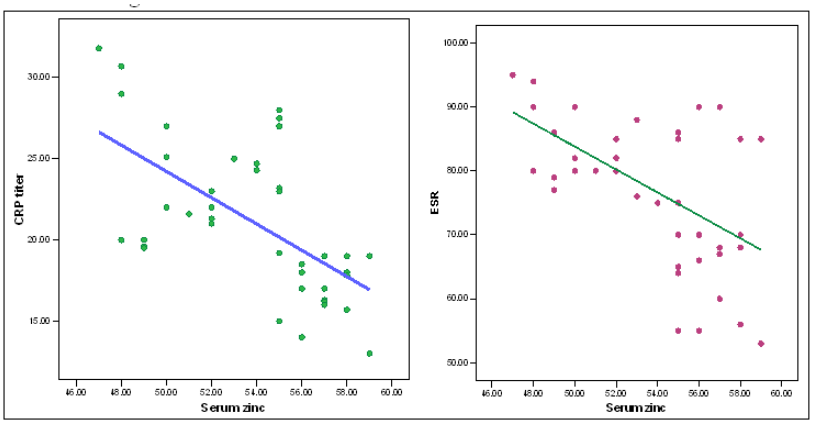

Fig-7: Correlation between serum zinc and serum CRP & ESR

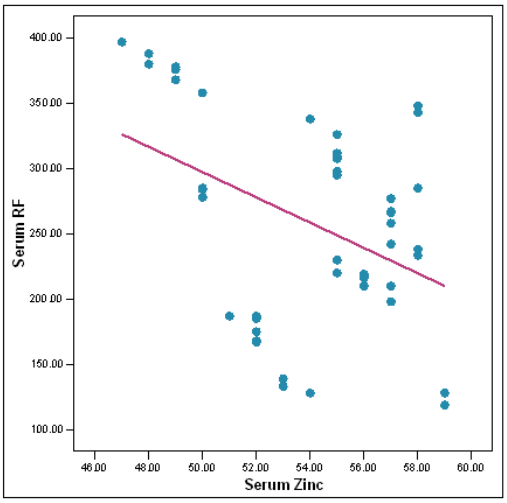

Fig-8: Correlation of serum zinc with serum RF

DISCUSSION

A total of 100 subjects were included in this cross sectional comparative study which was conducted in the Department of Biochemistry, Dhaka Medical College, Dhaka during the period from July, 2014 to June, 2015. Among the study subjects, 50 rheumatoid arthritis patients, diagnosed by new ACR criteria (2010), were considered as case that attended in the OPD of Physical Medicine department and admitted in the ward of Medicine department of DMCH, Dhaka with collaboration Biochemistry department. The rest 50 subjects considered as control that was taken from the attendance of the patients, hospital staffs and the staffs of the biochemistry laboratory. History of gouty

arthritis, traumatic arthritis, psoriatic arthritis, osteoarthritis, patients suffering from DM, CRF, autoimmune disease, hepatic dysfunction, taking nutritional supplements, recent history of diarrhea and dysentery, pregnancy and the subjects who refused to give informed written consent were excluded from the study. Serum zinc level were assessed in both groups and statistically compared. Again, correlation of serum zinc level was done with serum anti-CCPA, ESR, CRP and RF. In the present study, all the parameters in adult healthy subjects were within reference values. In this present study it was observed that most of the patients were in 3rd to 5th decade in both groups which were 62.0% and 56.0% in case and control groups respectively. The mean age was found 38.80±10.15 years in case groups and 35.32±6.59 years in control groups. The mean age difference was not statistically significant (p>0.05) between two groups. In our country, Jelly *et al.,* [9] found the mean age 36.9±9.97 years and 37.07±9.77 years in case and control groups respectively that were similar with the present study. Many researchers observed that rheumatoid arthritis most frequently occurred at the age between 30-50 years. A study was conducted by Badsha H *et al*., [20] where the mean age was found 42.2±12.3 years which was almost consistent with the present study. On the contrary, other studies regarding mean age conducted by Caglayan O *et al*., [21], Hadeal S *et al*., [22] were not consistent with the present study. In the present study, most of the subjects were female in both group (72% in group-A and 70% in group-B) where male-female ratio was 1:2.57 and 1:2.33 in group-A and group-B respectively. Many researchers found that rheumatoid arthritis were more common in female and it might be due to female hormone. Jamshidi *et al.,* [23] found that rheumatoid arthritis was six times more common in female than male which was consistent with the result of the present study. Vanhoof *et al*., [24] showed that 69.0% of patients with rheumatoid arthritis were female which was almost similar of the present study. Occupational and socio-economic status was also similar of other studies. It was observed in the present study that, almost two third (68.0%) subjects in group-A and 58.0% subjects in group-B were involved in the household works, not worked in outside. Another observation was also found in this study that, about 84.0% and 72.0% of the study subjects in group-A and group-B respectively were in poor class. The difference of occupational and socio-economic status between two groups was not statistically significant. Badsha H *et al*., [20] observed that about 56.0% patients were not worked in outside. Farooqi *et al.*, [25] observed that majority of the patients with rheumatoid arthritis were in poor class. In the present study it was observed that Erythrocyte Sedimentation Rate (ESR) was much higher in group-A (79.94±12.21) than group-B (9.92±1.99), p=0.001 which is statistically significant. A similar study was done by Taneja *et al*., [26], Caglayan O *et al*., [21]. Caglayan O noticed that the mean ESR was 59.6±26.6 mm/h which was higher than control and was consistent with this study [21]. Hasse and Rink, [10], Zoli *et al*., [8] both of them observed that decreased serum zinc level causes elevated production of pro-inflammatory cytokines like IL-1, IL-6, TNF-α which were responsible for the higher ESR level of the patients with rheumatoid arthritis than the control. Honkanen V. E *et al*., [27] also found the similar result which was consistent with the present study. Serum Anti-CCPA was found higher in case group (322.9±61.99) than in control group (18.46±3.70) which was highly significant P=0.001 (P<0.05) in comparison between two groups. In this study, CRP titer was higher in group A (21.02±4.43) than in group B (5.11±0.53) which was statistically highly significant P=0.001 (P<0.05). According to new diagnostic criteria (ACR/EULAR criteria 2010) Anti-CCPA is the hallmark of the diagnosis of early rheumatoid arthritis which has the specificity of 95%. Gambino R [28] observed high CRP titer in rheumatoid arthritis as because decreased serum zinc level causes elevated production of IL-1 that is responsible for the rapid synthesize of C- reactive protein by hepatocytes. Caglayan O, [21] also observed that mean serum CRP level (IU/L) in the patients with rheumatoid arthritis was high (41.2±39.7) than in control which was consistent with the present study.

In the present study, serum zinc level was observed decreased in group-A (56.14±8.64) than in group-B (90.38±14.03) which was highly significant statistically P=0.001 (P<0.05).A similar study was done by Taneja *et al.,* [26], Caglayan O *et al.,* [21] where they observed decreased serum zinc level which was consistent with the present study. Jelly *et al.,* [9] observed serum zinc level (µgm/dl) is higher (109±14.65) in control than in patients with rheumatoid arthritis (89.58±12.29) which was highly significant statistically P=0.000 (P<0.05). Zoli *et al.,* [8] suggested that decreased serum zinc level in rheumatoid arthritis was due to sequestration of zinc in the liver, pancreas and intestine due to cytokine induced metalloenzymes synthesis.In this study, a negative correlation was found in between serum zinc and serum anti-CCPA, serum CRP and serum ESR. It was observed that serum zinc had negative correlation with serum anti-CCPA (r= -0.704), P=0.001 which was highly significant statistically (P<0.05). Another observation was found that serum zinc had negative correlation with serum CRP (r= -0.613), P=0.001(P<0.05) and serum ESR (r=-0.544), P=0.001 (P<0.05). Both of this result was highly significant statistically. A similar study was conducted by Taneja *et al*., [26] where they found that a negative correlation of serum zinc between inflammatory markers serum C-reactive protein and ESR (r= -0.845) and (r= -0.846) respectively, which was consistent with the present study.

Limitations of the Study

Although the result of this study supports the hypothesis, there are some facts to be considered which

might affect the result. The following limitation encountered by investigator is to be kept in mind while reviewing the report.

- Because limitation of time and financial binding, the study was conducted with small sample size. So, it may not be adequate to represent the total population.
- This was a single hospital based study, so the result of the present study may not be representative.
- The sample was taken purposively, so there may be a chance of bias which can influence the result.
- Since the time period was very limited and there were other constrains the sample size could not be taken as desired.

CONCLUSION AND RECOMMENDATIONS

From the present study, it may be conclude that zinc has a major influence on the inflammatory response which occurs in the patients with rheumatoid arthritis. Decreased serum zinc level was associated with rheumatoid arthritis. So, for the evaluation of RA, all the biochemical parameters including serum zinc status should be sort out routinely for proper management as well as to reduce impending complications.

REFERENCES

1. Charpin C, Arnoux F, Martin M, Toussirot E, Lambert N, Balandraud N, Wendling D, Diot E, Roudier J, Auger I. New autoantibodies in early rheumatoid arthritis. Arthritis research & therapy. 2013 Aug;15(4):R78.
2. Wong R, Davis A, Badley E, Mohammed M. Prevalence of Arthritis and Rheumatic Diseases around the World: A Growing Burden and Implications for Health Care Needs. MOCA Health Report No. MOCA2010-02/007. 2010.
3. Lipsky L. Queueing Theory: A linear algebraic approach. Springer Science & Business Media; 2008 Dec 17.
4. Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. Lancet, 2010; 376(9746): 1094-1098.
5. Ahmed M, Haq SA, Islam MN, Banik SK, Alam MN. Burden of rheumatic diseases in a rural community of Bangladesh. Journal of Medicine. 2014 Oct 19;15(2):125-30.
6. Maret W, Sandstead HH. Zinc requirements and the risks and benefits of zinc supplementation. Journal of Trace Elements in Medicine and Biology. 2006 May 10;20(1):3-18.
7. Andreini C, Bertini I. A bioinformatics view of zinc enzymes. Journal of inorganic biochemistry. 2012 Jun 1;111:150-6.
8. Zoli A, Altomonte L, Caricchio R, Galossi A, Mirone LU, Ruffini MP, Magaro M. Serum zinc and copper in active rheumatoid arthritis: correlation with interleukin 1β and tumour necrosis factor α. Clinical rheumatology. 1998 Sep 1;17(5):378-82.
9. Jelly AA, Begum S, Ferdousi S. Serum zinc, copper and calcium levels in female rheumatoid arthritic patients. Journal of Bangladesh Society of Physiologist. 2012 Jul 10;7(1):18-22.
10. Rink L, Haase H. Zinc homeostasis and immunity. Trends in immunology. 2007 Jan 1;28(1):1-4.
11. Brown BG, Zhao XQ, Chait A, Fisher LD, Cheung MC, Morse JS, Dowdy AA, Marino EK, Bolson EL, Alaupovic P, Frohlich J. Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. New England Journal of Medicine. 2001 Nov 29;345(22):1583-92.
12. World Health Organization. The world health report 2002: reducing risks, promoting healthy life. World Health Organization; 2002.
13. Aiuti A, Biasco L, Scaramuzza S, Ferrua F, Cicalese MP, Baricordi C, Dionisio F, Calabria A, Giannelli S, Castiello MC, Bosticardo M. Lentiviral hematopoietic stem cell gene therapy in patients with Wiskott-Aldrich syndrome. Science. 2013 Aug 23;341(6148):1233151.
14. Doherty M, Ralston SH. Musculoskeletal disease. In: Nicki RC. Brian RW Stuart HR. (eds.). *Davidson's principles and practice of medicine* (21st Ed.). Edinburgh: Churchill Livingstone/Elsevier. 2010.
15. Haberland N, Chong E, Bracken H, WHO G. Married adolescents: An overview. Geneva: WHO. 2003 Dec 9.
16. Licitra L, Perrone F, Bossi P, Suardi S, Mariani L, Artusi R, Oggionni M, Rossini C, Cantu G, Squadrelli M, Quattrone P. High-risk human papillomavirus affects prognosis in patients with surgically treated oropharyngeal squamous cell carcinoma. Journal of Clinical Oncology. 2006 Dec 20;24(36):5630-6.
17. Smolen JS, Keystone EC, Emery P, Breedveld FC, Betteridge N, Burmester GR, Dougados M, Ferraccioli G, Jaeger U, Klareskog L, Kvien TK. Consensus statement on the use of rituximab in patients with rheumatoid arthritis. Annals of the rheumatic diseases. 2007 Feb 1;66(2):143-50.
18. Sarzi-Puttini P, Comi D, Boccassini L, Muzzupappa S, Turiel M, Panni B, Salvaggio A. Diet therapy for rheumatoid arthritis: A controlled double-blind study of two different dietary regimens. Scandinavian journal of rheumatology. 2000 Jan 1;29(5):302-7.
19. Danao-Camara TC, Shintani TT. The dietary treatment of inflammatory arthritis: case reports and review of the literature. Hawaii medical journal. 1999;58(5).
20. Badsha H, Kong KO, Tak PP. Rheumatoid arthritis in the United Arab Emirates. Clinical rheumatology. 2008 Jun 1;27(6):739-42.
21. Caglayan O, Aydog YS. Serum zinc and copper levels in rheumatoid arthritis. bone. 1997;7:9.

22. Al-Kuraya K, Novotny H, Bavi P, Siraj AK, Uddin S, Ezzat A, Al Sanea N, Al-Dayel F, Al-Mana H, Sheikh SS, Mirlacher M. HER2, TOP2A, CCND1, EGFR and C-MYC oncogene amplification in colorectal cancer. Journal of clinical pathology. 2007 Jul 1;60(7):768-72.
23. Jamshidi AR, Banihashemi AT, Roknsharifi S, Akhlaghi M, Salimzadeh A, Davatchi F. Estimating the prevalence and disease characteristics of rheumatoid arthritis in Tehran: a WHO-ILAR COPCORD Study (from Iran COPCORD study, Urban Study stage 1). Medical journal of the Islamic Republic of Iran. 2014;28:93.
24. Van Hoof A, Frischmeyer PA, Dietz HC, Parker R. Exosome-mediated recognition and degradation of mRNAs lacking a termination codon. Science. 2002 Mar 22;295(5563):2262-4.
25. Farooqi M, Saleemuddin M, Ulber R, Sosnitza P, Scheper T. Bioaffinity layering: a novel strategy for the immobilization of large quantities of glycoenzymes. Journal of biotechnology. 1997 Jul 4;55(3):171-9.
26. Taneja SK, Mandal R. Assessment of mineral status (Zn, Cu, Mg and Mn) in rheumatoid arthritis patients in Chandigarh, India. Rheumatology Reports. 2009 Jul 23;1(1):5.
27. Honkanen V, Konttinen YT, Mussalo-Rauhamaa H. Vitamins A and E, retinol binding protein and zinc in rheumatoid arthritis. Clinical and experimental rheumatology. 1989;7(5):465-9.
28. Gambino R, Desvarieux E, Orth M, Matan H, Ackattupathil T, Lijoi E, Wimmer C, Bower J, Gunter E. The relation between chemically measured total iron-binding capacity concentrations and immunologically measured transferrin concentrations in human serum. Clinical chemistry. 1997 Dec 1;43(12):2408-12.