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RESEARCH ARTICLE

SEX DIFFERENCES IN TRACE METALS AND DISEASE ACTIVITY IN PATIENTS WITH RHEUMATOID ARTHRITIS.

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Abstract

Objectives: Rheumatoid arthritis (RA) is chronic autoimmune disease marked by tissue inflammation and joint destruction. Sex differences and essential element derangement in the incidences of RA are well described. **Methods:** The present study aims to determine serum mineral level, superoxide dismutase (SOD) activity and disease activity score (DAS) in female and male RA subjects and association of minerals with disease activity in both the sexes.
Results: Female RA patients had significantly reduced serum Zn as compared to male RA subjects. Females had non significantly low values for copper, magnesium and phosphorous as compared to male RA subjects. SOD and DAS were significantly increased in female RA subjects. The RA subjects had higher levels of serum zinc, copper and lower levels of serum Mg when compared with reference values. However none of them were found to be associated with disease activity.
Conclusions: The results suggest derangement of minerals in RA where mineral evaluation and supplementation especially of magnesium in RA patients would be helpful.

Introduction:-

Rheumatoid arthritis (RA) is chronic inflammatory autoimmune disorder of unknown etiology affecting various symmetric joints of the body. Like other autoimmune diseases RA is also more prevalent in females as compared to males[1-4]. Female to male ratio in RA is 3:1[5]. The differences in over occurrence and development of aggressive disease in females is not clear but genetic and hormonal factors are suggested to be involved[6-11].

Several observational studies suggest that women with RA have worst disease as compared to men[12-16] .
In recent studies RA seems to have derangement of mineral content like Mg, Zn, Cu and P. Their optimum concentration is required for normal functioning of the body. However alterations in level of these trace minerals as Mg[17], Zn[18] and Cu (Copper) have been implicated in pathogenesis of RA as they are the co-factor of important enzymes involved in collagen and bone metabolism, the antioxidant defense system[19] and the immune system[20]. The development and progression of RA was suggested due to marginal deficiencies of Zn and Cu based on their

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serum levels[21,22]. Many of these trace elements are present in bones as iron, copper, zinc, manganese, fluoride, stroncium and boron[23]. As the changes in the concentration of trace elements has been linked to inflammatory response therefore the present study was undertaken to analyze i) The concentration of Zn, Cu, Mg and P in female and male RA subjects along with activity of superoxide dismutase (SOD) and disease activity score (DAS-28-CRP). These may help in determination of possible roles of these in disease activity of female and male RA patients.

Materials and Methods:-

The study was started after approval from Institutional Ethical Committee and written informed consent was obtained from all the participants. 46 samples (31 females, 15 males) were randomly collected from the OPD of Orthopaedics from different centers during the study period. Patients were recruited with active RA who fulfilled 4 or above criteria of American College of Rheumatology (ACR)[24].

Clinically the patients presented symmetric arthritis with complaints of severe multiple joint pain along with morning stiffness (>1hr) of joint, presence of rheumatoid nodules along with radiographic changes like erosion, swelling (>3 joint especially phalanges), multiple joint involvement and deformity of peripheral joints (metacarpophalangeal (MCP) and proximal interphalangeal joint (PIP)) and decreased range of motion. The patients did not had any renal disease and were non hypertensive. Blood was drawn from overnight fasting patients for all the analysis.

Laboratory Analysis;-

The laboratory analysis for minerals was done by commercial kits from Coral clinical system (The Tulip group, India). Serum level of magnesium were estimated by Calmagite Method by using the UV- 1800 SHIMADZU UV Spectrophotometer at the wavelength of 510nm. Serum level of the Copper and Zinc were measured by colorimetric method by using the UV- 1800 SHIMADZU UV Spectrophotometer at the wavelength of 580nm, 570nm respectively. Serum Phosphorous level by Molybdate UV Method by using the UV- 1800 SHIMADZU UV Spectrophotometer at the wavelength of 340nm. SOD estimation was done according to Mishra and Fridovich, 1972[25]. DAS-28 was measured according to counts of affected joint count and final value was calculated using CRP values.

Statistical Analysis:-

The data was analyzed with Student's independent t- test. The correlation of change in biochemical parameters was done by Pearson correlation analysis. A two-tailed (α=2) $p<0.05$ was considered to be statistically significant. Graphpad Prism (version 3.0) and SPSS-15 were used for the analysis.

Results:-

The study was done on 46 RA patients (31 females and 15 males). Their was no significant difference in the BMI of female and male patients. Female patients and male patients had significant difference in the mean age. They had reduced levels of serum zinc (131.48μmg/dl Vs 138.32μmg/dl $p<0.05$), serum copper (153.65mg/dl Vs 157.06mg/dl), serum magnesium (1.006mEq/l Vs 1.106mEq/l) and serum phosphorous (6mg/dl Vs 6.166mg/dl) as compared to male participants (table 1). Both female and male RA subjects had reduced levels of serum magnesium as compared to reference values. The activity of super oxide dismutase (SOD) and disease activity score (DAS-28-CRP) were significantly high in female patients as compared to males (table 1).

Intercorrelation between different parameters studied showed no correlation of any of the essential minerals tested with DAS-28-CRP or SOD in neither females (Table 2) nor males (table 3). However female patients showed significant negative correlation ($r=-0.434$; $p<0.05$) between phosphorous and copper (table 2).

Table 1:- shows the parameters analyzed in female and male subjects. P-phosphorous; SOD-Superoxide dismutase; DAS-28-CRP-Disease activity score-28-C-reactive protein.

	Females N=31	Males N=15	Reference range
Age	33.60±1.817	43.53±2.74*	
BMI	21.98±0.76	22.96±0.72	
Zinc (*mg/dl)	131.48±1.91	138.32±1.96*	75-120*mg/dl
Copper(*mg/dl)	153.65±3.47	157.066±2.46	70-140*mg/dl
Mg (mEq/l)	1.006±0.686	1.71±0.051	1.3-2.5mEq/l
P (*mg/dl)	6.006±0.147	6.11±0.1002	2.5-50(*mg/dl)
SOD (u/mg protein)	1330.88±22.73	1024.60±53.91*	
DAS-28-CRP	5.131±0.216	4.73±0.119	

Table 2:- Intercorrelation between different parameters in female RA patients. SOD-Superoxide dismutase. DAS-28-CRP-Disease activity score-28-C-reactive protein. * shows significant correlation ($p<0.05$).

	Zinc	Copper	Magnesium	Phosphorous	SOD	DAS-28-CRP
Zinc	1					
Copper	-0.021	1				
Magnesium	-0.117	-0.119	1			
Phosphorous	0.212	-0.434*	0.007	1		
SOD	-0.271	0.150	-0.029	-0.211	1	
DAS-28-CRP	0.098	0.256	0.077	-0.215	-0.139	1

*Significant values have $p<0.05$.

Table 3:- Intercorrelation between different parameters in male RA patients. SOD-Superoxide dismutase.

	Zinc	Copper	Magnesium	Phosphorous	SOD	DAS-28-CRP
Zinc	1					
Copper	0.105	1				
Magnesium	-0.252	-0.252	1			
Phosphorous	0.260	-0.457	0.04	1		
SOD	0.232	0.105	0.138	0.422	1	
DAS-28-CRP	0.479	-0.044	0.336	0.001	0.011	1

DAS-28-CRP-Disease activity score-28-C-reactive protein

Discussion:-

There was no significant difference in the BMI of female and male patients. Females have more aggressive and painful disease than males. As female patients in our study had less average age than males suggesting early onset of RA in them as compared to males. There are differences in functional capacity in female and male subjects with RA where females have more functional impairment than males[26]. In our study also DAS-28-CRP is higher in females as compared to males. These differences may be due to general strength of bones and muscles, bone mineral density (BMD), hormones etc. However the widely used measure of function, the health assessment questionnaire (HAQ), is cross-sectionally higher (worse) in women[26].

In the present study, we found decreased level of serum magnesium in female and male RA subjects as compared to reference range, though no significant difference was observed between the two sexes in serum magnesium levels. Chronic inflammatory conditions in RA may alter the levels of magnesium and possible mechanism of reduced magnesium may be due to chronic inflammation and autoimmune injury[17,27,28]. Our results are in accordance with[17,27-29] suggesting that RA, is associated with serum magnesium disturbances [28]. Mg is one of the essential nutrient of the body and studies suggest its role in reducing chronic inflammation[30]. Decreased Mg level is considered as marker for RA[17]. Magnesium is an activator of sodium potassium ATPase, is antiarrhythmic and is associated with cardio vascular disease susceptibility[31-32]. Inflammation trigerrs its deficiency in animal models. In humans, low serum magnesium concentrations have been associated with high C-reactive protein (CRP) levels[33].

The level of phosphorous and copper was non-significantly higher in male RA patients as compared to females. In female RA patients, phosphorous showed inverse correlation with copper ($r = -0.434$; $p<0.05$). There is strong association between elevated phosphorous and Ca and phosphorous products and the development of calciphylaxis. Phosphorous influences a number of pathways involved in vascular calcification. It also has a role in induction of differentiation of vascular smooth muscle cells into osteoblast-like cells capable of extraskeletal mineralization which is important process in development of vascular calcifications. Thus phosphorous may have a role in augmenting inflammation.

Both female and male RA patients had higher serum copper levels as compared to reference values. RA patients are shown to have high levels of copper[34,35]. Their levels have been shown to increase in all inflammatory processes including RA. Ceruloplasmin, a copper containing enzyme is found to be significantly elevated in inflammatory conditions and has anti-inflammatory activity[36-37]. Our findings are consistent with Scudder et al 1978[38] and Tuncer et al 1999[39]. Hypercuperemia associated with inflammatory response may be due to oxidative stress[40] as positive correlations were found between serum Cu levels and inflammatory markers as serum CRP and ESR in RA patients[41]. Yang et al, 2016[42] have reported that RA patients living where farm soil has high copper had higher WBC count, ESR, DAS-28 and platelet count than people living in areas with low levels of copper. Cu is an environmental bioelement having key role in the cell's physiology. It is cofactor or component of the enzymes, participating in anti-oxidative processes, or in detoxification of oxygen free radicals. Cu complexes were effective in treating arthritis as Cu complexes have anti-inflammatory properties. Antiarthritic drugs in their active forms are complexed with copper[35]. The hypercupreuria was also suggested to be the outcome of dyslipedemia[43] or inflammation, as the cytokines have been reported to enhance the release of Cu thioneins during the oxidative burst of polymorphonuclear cells[44]. As many studies have reported higher levels of copper in active RA, therefore copper has been suggested to be used as an additional biochemical biomarker for RA[45].

In our study the levels of zinc were significantly higher in males as compared to females. This clearly shows that RA patients are not deficient in Zinc or copper[19]. However, Zoli et al., 1998[46] reported lower zinc levels in RA patients. As zinc is considered anti-inflammatory with studies showing negative correlation between zinc and levels of IL-1β and TNF-α. Mierzecki et al, 2011[18] have reported nonsignificant but higher levels of zinc in serum of RA patients. Though zinc levels should have been lower considering the role of proinflammatory cytokines as IL-1 and TNF- α which inhibit albumin synthesis in liver and lower their zinc-binding capacity, which should in turn reduce the plasma zinc levels. However lower values of zinc in other studies may be due to pharmacological treatments or other effects which also need to be considered. Serum zinc levels have been shown to decrease during acute-phase response of inflammation and with treatment with NSAIDS[44]. Probably alterations in inflammation may have some role in the levels of essential minerals.

Inflammation may be the primary cause for systemic alterations in the levels of minerals and enzymes which further modulate acute phase plasma proteins[47]. The study Chavan et al. 2015 by[29] have shown negative correlation between serum magnesium with TC, triglycerides, LDL-c and positive correlation with HDL-c. Thus lower serum magnesium may be associated with worsened lipid profile and increased CVD risk of RA patients.

In our study SOD activity is significantly increased in female RA patients as compared to males. The treatment of Methotrexate (for control of RA) has been reported to increase Zn-SOD activity in rats[48-49].

Increased activity of SOD[50-51] may also be due to increased O_2^- production by hyperactive cells[52-53]. Higher SOD levels may be an after effect to nullify excessive free radical production. Post treatment the antioxidants are increased which lead to lower plasma MDA and increased total antioxidant capacity (TAC)[54,55].

RA is associated with serum mineral disturbances and oxidative stress. Our results show that male and female patients of RA neither differ much in copper, phosphorous and zinc levels nor the minerals are associated with DAS. Serum magnesium was lower in both sexes in RA subjects. Serum magnesium and serum copper may be useful biomarker for RA.

Compliance with Ethical Standards:-

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Ethical Approval: The study was approved by Institutional ethical committee and written informed consent was obtained from all the subjects.

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