Research Article

Serum zinc, copper and selenium level in inflammatory bowel disease patients and their relation with metabolic bone disease

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Abstract

Bone metabolism changes in patients with inflammatory bowel disease (IBD) awoke a growing interest in the past few years mostly because of their high prevalence, with estimations around 40-50% for osteopenia and 5-30% regarding osteoporosis. Contributing factors including inadequate nutrition, corticosteroid, and decreased physical activity. Trace elements play an important role in the growth development and maintenance of bones. The aim of our study was to assess the Bone mineral indexes and serum Zinc, Copper, Selenium levels in Inflammatory Bowel Disease patients and their correlation with bone mineral density. One hundred newly diagnosed patients of Inflammatory Bowel Disease and 50 healthy Controls of both gender ranging in age from 19-50 years were included in the study. Fasting blood samples were processed for all baseline parameters. Serum Zinc, Copper and Selenium assessed by Atomic Absorption Spectrophotometry. The subjects were evaluated for Bone Mineral Density (g/cm2) using Dual Energy X-ray Absorptiometry scan and T score was calculated to assess Osteoporosis. Student's unpaired t-test, one way ANOVA and Pearson correlation tests were used for statistical analysis. Inflammatory Bowel Disease patients had significantly lower Bone Mineral Density than the Controls. Bone Mineral Density values were not different between the subtypes Crohn's Disease and Ulcerative Colitis. Though Ulcerative Colitis and Crohn's Disease patients had significantly lower Bone Mineral Density than the Controls. Significantly Low Zinc and selenium level was observed in Inflammatory Bowel disease patients however Copper was found significantly high. Zinc, Copper and Selenium level was significantly correlated with Bone Mineral Density(r=0.24,-0.25,0.22). Patients with Inflammatory Bowel Disease are more prone to develop metabolic bone disease. Along with other nutrients supplement Zinc, Copper and Selenium should be added to prevent bone loss as well as oxidative stress in Inflammatory Bowel Disease patient.

Keywords: Inflammatory Bowel Disease, Ulcerative Colitis, Crohn's Disease, Osteopenia, Osteoporosis.

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INTRODUCTION

Inflammatory bowel disease (IBD) classically covers Crohn's disease (CD) and ulcerative colitis (UC). Both are chronic systemic inflammatory diseases, affecting mainly the gut, but complicating extra intestinal manifestations (EIMs) are found in about 40% of the patients depending on the different populations studied[1] The disease causes inflammation of the digestive tract which is traditionally found at opposite ends of the disease spectrum[2]. UC is exclusively restricted to the large bowel, while CD can virtually affect any segment in the digestive tract. Disease onset occurs typically during young adulthood (25–35yr), although 20–25% of cases are diagnosed during childhood[3]. Systemic nature of IBD also causes extra intestinal manifestations including ankylosing spondylitis, pyodermagangrenosum, erythema nodosum, iritis, uveitis, episcleritis, primary sclerosing cholangitis, venous thromboembolism, avascular

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necrosis, and ischemic arterial events. Osteopenia and Osteoporosis are two of the more common extra intestinal symptoms with a general consensus that IBD patients are at a significantly higher risk of developing metabolic bone disease and low Bone Mineral Density (BMD) than the healthy subjects[4]. The relative risk of fracture in IBD patients has been estimated to be 40% higher than in general population[5,6]. The pathogenesis of Osteoporosis and the mechanisms of bone loss in IBD patients have not been clearly characterized. Bone loss seems to be multi factorial that is disease itself, disease activity, malnutrition in general, malabsorption of the nutrients important for the development of bone such as Calcium, Phosphorus, Vitamin D etc.[7] Vitamin and Mineral deficiencies tend to be a real problem for people with IBD. Several other factors also contribute to these Vitamin and Mineral deficiencies, including inflammation, diet, and the treatment for IBD itself. The use of Vitamins and Minerals by the body is complex, with many nutrients requiring the presence of other Vitamins to facilitate their uptake and use by the body. If the body is not receiving even one Vitamin it needs, the end result could be a chain reaction affecting many other Vitamins and Minerals[8]. Trace elements are present in very small amounts in the body (i.e., nanograms or parts per million) including zinc, copper, and selenium. Protein-energy malnutrition most often occurs with active, severe IBD. However, micronutrient deficiencies can occur even with disease that is relatively mild or in remission. Multiple simultaneous deficiencies in micronutrients are more common in patients with Crohn's disease (CD), especially those with fistulas, strictures, or prior surgical resections of the small bowel[9]. As a nutrient, Zinc, Copper and Selenium may have some role in bone development as it is needed for osteoblastic activity, collagen synthesis and alkaline phosphatase activity[10] A study has shown a positive relation between Zinc intake and Bone Mineral Content (BMC) in children[11] There is paucity of data regarding role of Zinc, Copper and Selenium in IBD and its association with bone metabolism for Indian population. The aim of our study was to investigate serum Zinc, Copper and Selenium level in IBD patients and correlate them with bone Mineral indexes.

MATERIAL AND METHODS

The study was carried out in the Department of Biochemistry in association with Department of Gastroenterology at S.M.S. medical college and Hospital, Jaipur. Hundred newly diagnosed patients of IBD of both gender (57% were male and 43% were female) ranging in age from 19-50 years were included in the study. IBD was diagnosed on the basis of history, clinical, endoscopic and histologic findings. The exclusion criteria for the selection of IBD patients were Hepatic or renal disease (creatinine> 1.5 mg/ dl), Thyroid and parathyroid diseases, Diabetes mellitus, Previous corticosteroid use for any reason(for more than 3 months), Inflammatory joint disease (ankylosing spondylitis, rheumatoid arthritis etc.),Treatment for Osteoporosis (bisphosphonates, calcium, Vitamin D, fluoride, calcitonin and hormone replacement therapy) and severe neurologic disease. Fifty age and sex matched healthy subjects in which 62% male and 38% female were recruited as comparison group. An informed written consent was taken by all the recruited subjects. Blood samples were collected from all the subjects in plain vials by venepuncture after 12 hour fasting and processed for serum Glucose, Total Cholesterol, Urea, Creatinine, Total Protein, Albumin, Alkaline Phosphatase (ALP), Aspartate aminotransferase (AST), Alanine aminotransferase(ALT), Calcium, Phosphorus using standard kits on Olympus 400 Autoanalyzer in the Central Clinical Biochemical Laboratory, S.M.S. Medical College, Jaipur. Serum Zinc, Copper and Selenium was estimated on Atomic Absorption Spectrophotometer (Model-AAS 4141 by ECIL). The Bone Mineral Density (g/cm2) was measured within 1 week of diagnosis using the method of Dual Energy X-ray Absorptiometry (DEXA) scans of the lumber spine (L2-L4) using a Hologic QDR 4500 W scanner (Hologic 800-321-4659,Model no- Explorar). Lumber T scores were calculated using locally validated manufacturer's reference ranges. IBD patients were further segregated on the basis of T score. Osteopenia was defined as a value for BMD between -1.0 to -2.5 standard deviation below the young adults mean (i.e. score between -1.0 and -2.5). Osteoporosis was defined as a value for BMD that was -2.5 standard deviation between or more below the young adults mean (i.e. T score less than -2.5)[12].

Statistical analysis

All the parameters were expressed as mean and standard deviation (SD). To compare a continuous variable between groups, the Student's unpaired t-test was performed. Analysis of Quantitative data between a qualitative variable with more than two subgroups was done using One-way ANOVA. Tukey's Post Hoc test was then used for observations between individual groups of patients if p-value of ANOVA was statistically significant ($p<0.05$).Pearson correlation was used for univariate association of Zinc, Copper and Selenium with BMD. P value <0.05 was considered statistically significant.

RESULTS

Table 1: The levels of Serum Zinc, Copper, Selenium and Bone Mineral indexes in IBD subjects and Controls

Character	Mean ± SD (range)				P1	P2	P3
	Control	IBD	UC	CD			
Number	50	100	92	9			
T –score	-0.23±1.04 (-2.6-3.4)	-1.71±1.32 (-4.8-1.5)	-1.7±1.35 (-4.8 – 1.5)	-1.35±0.88 (-3.1 - -0.7)	0.0001	0.0001	0.004
BMD(g/cm^2)	1.01±0.18 (0.54-1.46)	0.87±0.18 (0.54-1.224)	0.87±0.18 (0.543-1.224)	0.86±0.14 (0.751-1.001)	0.0007	0.001	0.02
Zinc (µg/dl)	78.96±10.44 (64.09-104)	64.89±12.19 (40.8-105.20)	65.22±12.47 (40.8-105.2)	60.72±6.80 (54.2-72.1)	0.0001	0.0001	0.0001
Copper (µg/dl)	87.49±15.40 (69.35-132)	120.24±25.60 (70-165.8)	118.99±25.43 (70-165.8)	123.31±23.46 (89.8-165.8)	0.0001	0.0001	0.0001
Selenium (µg/dl)	13.65±1.90 (10.7-17.39)	12.30±1.78 (9.6-17.1)	12.34±1.81 (9.6-17.1)	11.61±1.24 (9.6-12.9)	0.01	0.01	0.01

P1-IBD vsControl, P2 UC vsControl, P3- CD vsControl

*(p < 0.05 significant); ** (P < 0.01 very significant);*** (P<0.001: highly significant) and Rest not significant (p>0.05).

Table 2: Serum Zinc, Copper, Selenium and Bone Mineral indexes in Normal, Osteopenia and Osteoporosis groups of IBD subjects

Character	Control	T score >-1 Normal	T score(-1 to -2.5) Osteopenia	T score<-2.5 Osteoporosis	F	P
Number	50	27	45	28		
BMD(g/cm^2)	1.01 ± 0.18 (0.54-1.46)	0.97±0.21 (0.558-1.224)	0.87±0.12 (0.77-1.17)	0.74±0.15 (0.568-1.175)	14.97	0.000
Zinc (µg/dl)	78.96±10.44 (64.09-104)	68.88±13.16 (53.1-89.1)	65.30±12.58 (40.8-105.2)	59.90±8.36 (40-80)	12.09	0.000
Copper (µg/dl)	87.49±15.40 (69.35-132)	108.16±27.39 (70-165.8)	123.07±22.52 (72.8-165.8)	127.84±25.29 (70-159.1)	81281.17	0.003
Selenium (µg/dl)	13.65±1.90 (10.7-17.39)	12.84±1.84 (9.6-17.1)	12.24±1.74 (9.4-17.3)	11.78±1.69 (9.8-17.1)	394.62	0.05

Comparison was done using ANOVA (Analysis of variance test) and Tukeys, p< 0.05 indicates that groups are responsible for variance in the measured variable and is highly significant and Rest are not significant (p>0.05).

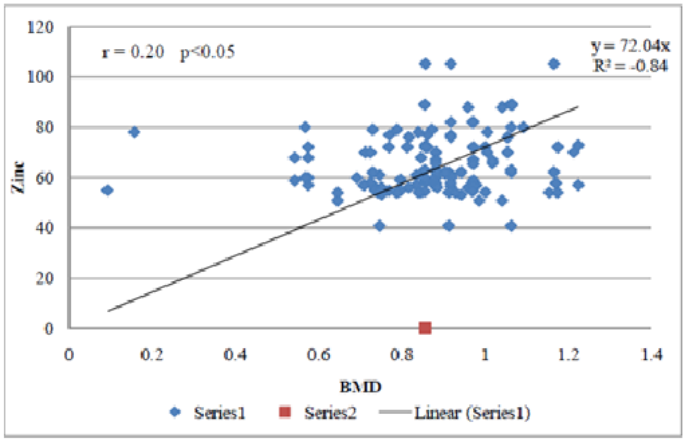

Figure 1: Correlation between Zinc and BMD

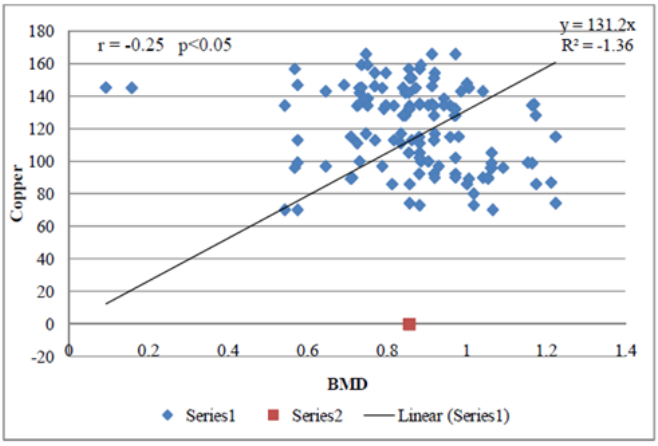

Figure 2: Correlation between Copper and BMD

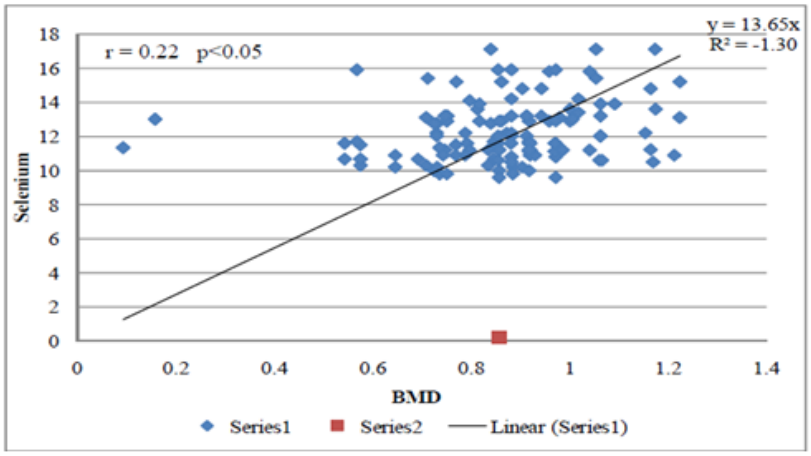

Figure 3: Correlation between Selenium and BMD

The mean age of the 100 IBD patients was 37.72 ± 12.40 years at the time of diagnosis. No patient had family history of IBD. The mean T-score of the patients with IBD was significantly low compared with the Controls (p=0.001). Difference against the Control group remained significant when the Cronhs Disease and Ulcerative Colitis patients were compared separately (Table 1). The mean lumbar spine BMD values and serum Zinc/Copper, Selenium level of the patients with IBD was significantly low as compared with the Controls (p<0.05).The mean lumbar spine BMD and serum Zinc and Selenium levels of the Cronhs Disease and Ulcerative Colitis groups were also significantly low however Copper was significantly high as compared to Control group (p<0.05). As T score is used as an indicator of metabolic bone disease. Further on the basis of T score we distributed the patients in Normal (T>-1), Osteopenia (T=-1 to -2.5) and Osteoporosis (T<-2.5) groups[12]. Among 100 IBD patients we observed that 27% IBD patients had Normal bone density, 45% were Osteopenic and 28% had Osteoporosis (Table 2). Our data demonstrated a high prevalence of Osteoporosis and Osteopenia as compared to Controls. We could confirm evidence that IBD patients are more prone to the problem of Osteoporosis and Osteopenia (73)%. The BMD of Osteoporosis group was found least, the mean value was 0.74±0.15 g/cm2. In Control group BMD was normal 1.01±0.18 g/cm2. Using ANOVA mean level difference of BMD was found highly significant among Control, Normal T score, Osteopenic and Osteoporotic group (p=0.000). Further on applying tukeys test with in Control and Normal, Control and Osteopenic, Control and Osteoporotic, Normal and Osteopenic, Normal and Osteoporosis and Osteopenic and Osteoporosis groups, BMD was highly statistically significant (p<0.0001). Serum Zinc and Selenium was lowest in Osteoporotic group while Copper was highest in Osteoporotic group and mean level difference of Zinc, Copper and Selenium among all the groups were statistically significant (p<0.0001).On applying tukeys test for inter group comparison only normal and osteoporotic group were found to have significant mean difference in serum Zinc, Copper and Selenium levels (p<0.05) however the mean difference between normal and osteopenic group for Copper is also found significant (p < 0.05). Serum Zinc and Selenium was significantly positively correlation with BMD (r=0.24, r=0.22, Fig 1,3) while BMD was negatively correlated with Copper (r=-0.25, Fig 2).

DISCUSSION

Bone metabolism changes in patients with IBD have drawn increasing attention in recent years.Osteoporosis is a global health problem that will become increasingly important as individuals live longer and the World's population continues to increase in number[13,14]. Fragility fractures, the hallmark of Osteoporosis, are a major cause of morbidity and mortality[15,16]. Nutrients and lifestyle plays an important role in bone health. Patients with IBD reveal various malnutrition status and in many studies deficiency of trace elements have been reported as characteristics feature of malnutrition[17]. Underlying mechanism postulated as causing Mineral deficiency in IBD include inadequate dietary intake decreased absorption, increased requirements and increased losses[18]. Zinc is an essential transition metal in humans, playing a catalytic, structural and regulatory role in the biological system. Zinc is abundant in bone tissue and is needed to maintain Bone Mineral Density and bone metabolism. Every step of bone metabolism utilizes Zinc, and its deficiency is implicated in Osteoporosis[19]. The organic matrix of bone is comprised of proteins that require adequate amounts of Zinc for optimal function. Zinc acts as a cofactor for osteoblast activity during bone formation and is required for maintaining peak bone density and reducing the risk of age-induced Osteopenia or fracture.

Recent evidence demonstrates that Zinc may act as a local regulator of bone cell formation by stimulating the proliferation and differentiation of osteoblasts while at the same time inhibiting osteoclast differentiation[20]. Low plasma Zinc is common in patients with CD and may be associated with clinical manifestations such as acrodermatitis, decreased activity of Zinc-dependent enzymes like thymulin and metallothionein, reduction in muscle Zinc concentration. Moreover, Zinc absorption is impaired and fecal Zinc losses are inappropriately high. Zinc deficient adolescents with CD grow and mature normally when Zinc deficiency is treated[21]. Zinc is an essential mineral, required for catalytic activity of ≈100 enzymes, including metalloproteinases, and is also important in immune function, protein and collagen synthesis, and wound healing. Zinc is absorbed along the length of the small intestine by a poorly characterized transport mechanism ((zinc is transported in part by albumin), but is also excreted in intestinal and pancreatic secretions. Zinc deficiency is thought to be relatively common in patients with chronic diarrhea, malabsorption, and hypermetabolic states (sepsis, burns)[22]. Zinc has been shown to be decreased in neutrophils both in patients with active Chrons Disease and those with Ulcerative Colitis. Since oxidative stress is increased in the inflamed mucosa. Zinc can also act as a cofactor for pufa delta-6-desaturase regulating the metabolism of arachidonic acid and the synthesis of eicosanoids. In addition Zinc has also been implicated in the cell mediated immunity response in the inflamed mucosa[23]. Sturnilo *et al* observed a highly significant correlation between plasma zinc and albumin suggests a possible role of plasma-binding alterations in the depressed plasma zinc levels in patients with IBD, this is similar to our study[24]. When serum Copper level was compared in all subjects it was observed that in IBD group Cu level was high as comparison to Controls. The mean difference of Copper in among the groups were highly statistically significant (p=0.001) and the mean level difference of Copper in IBD and Controls was found statistically significant. The possible mechanism of high Copper level in IBD subjects would be an excess in total tissue Copper, resulting in elevated nonspecific bound Copper which may, by itself, catalyze free radical formation and increase the oxidative stress of mucosa. Serum copper levels are often increased in patients with IBD and, during inflammation, may be accompanied by increased ceruloplasmin (the carrier molecule for copper) levels. Excess copper may increase oxidative stress in the colonic mucosa resulting in a continuous cycle of inflammation in IBD.Since free radicals can induce cell membrane peroxidation reaction with arachidonate and potentiate the synthesis of eicosanoids. The presence of both low Zinc levels and Copper excess may be of relevance in perpetuating the inflammatory processes in IBD[25]. The mean value of Serum Selenium in IBD subjects as compared to Controls was significantly low. Selenium behaves both as an antioxidant and anti-inflammatory agent. This is because Selenium in its antioxidant role, notably as glutathione peroxidase (GPX), can reduce hydrogen peroxide, lipid and phospholipid hydroperoxides, thereby dampening the propagation of free radicals and reactive oxygen species; reduce hydroperoxide intermediates in the cyclo-oxygenase and lipoxygenase pathways diminishing the production of inflammatory prostaglandins and leukotrienes; and modulate the respiratory burst, by removal of hydrogen peroxide and reduction of superoxide production[26]. In IBD, oxidative stress plays a major role in disease pathogenesis. GPX are Selenium dependent and some of the most important antioxidant enzymes in humans and therefore it is possible that selenoproteins (SNPs) in *GPX1* may be involved in modulating several diseases. GPX1 is a ubiquitous enzyme with antioxidant properties, and plays an important role in antioxidative defense and therefore a dysfunction of *GPX1* might be involved in CD pathogenesis[27]. Bone is highly dynamic and it undergoes constant remodelling throughout life. The remodeling involves coupled resorption of existing bone and the formation of new bone[28]. Essential trace minerals such as copper and manganese are required along with zinc for the maintenance of healthy bone tissue. These minerals are involved in the formation of the bone framework structure contributing to the organic component of the osseous matrix. The hard mass characteristic of healthy bone is formed by inorganic minerals such as Calcium and Phosphorous, a component typically referred to as the "mineral mass." The structural framework around which the "mineral mass" deposits is termed the "organic bone matrix." The organic matrix is comprised of proteins that require zinc, manganese and copper as essential co-factors for enzymes involved in their synthesis[29]. Deficiency in zinc will lead to bone growth retardation and osteopenia due to insufficient bone mineral mass[30]. Many of the trace elements which have essential roles in animals, such as zinc (Zn), manganese (Mn), copper (Cu) and ALP are required for the growth, development and maintenance of healthy bones. The aim of our study was to assess the relationship among the serum Zinc, Copper and Selenium level and bone Mineral indexes of IBD patients. In our study IBD patients had significantly lower BMD values than the Controls. Further comparing the values of BMD in IBD types we found that BMD values were not different between the CD and UC patients, Though UC and CD patients had significantly lower BMD values than the Controls. CD patients had lower

mean serum Zinc and Selenium level than UC patients, but the difference was insignificant. Metabolic bone disease develops silently in these patients, the origin of which is probably multi factorial: lack of physical activity, deficiencies of Calcium, Phosphorus, Vitamins and other micronutrients (Zinc, Copper and Selenium). Intestinal malabsorption has been intuitively linked to the pathogenesis of bone loss in IBD and celiac disease patients[31]. When we further subdivided the IBD patients among Normal, Osteopenic and Osteoporotic group and compared them with Controls we found that in Osteopenic group 50% patients had altered serum Zinc, Copper and Selenium level while in Osteoporotic group 80% patients had altered serum trace elements level. Further serum Zinc and Selenium level was positively correlated with Bone Mineral Density (r=0.24, r=0.22) while copper is negatively correlated with Copper (r=-0.25) in Inflammatory Bowel Disease patients. Thus correction of Zinc, Copper, Selenium deficiency by specific intervention will have beneficial effects on Osteoporotic activity in IBD patients.

CONCLUSION

Patients with IBD are more prone to develop metabolic bone disease.Bone fractures occur without clinical signs more frequently in IBD compared to other populations. We need a much more accurate risk assessment system to predict fractures for proper therapy in this young population with a life-long chronic inflammatory disease. Nutrition plays an important role in the management of patients with IBD. In both UC and Crohn's disease, patients can have excessive stool and blood losses develop high output fistulas that may require supplement minerals. To minimize risks of metabolic bone disease, patients with IBD should be given Zinc, Copper, Selenium supplements along with other nutrients to prevent bone loss.

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