Original Research Article

Study of hematological parameters in patients with diabetic retinopathy

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Abstract

Bonedemineralization is a significant problem in Inflammatory Bowel Disease. 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 relationship between the serum Zinc level and the bone Mineral indexes in Inflammatory Bowel Disease patients. Forty two newly diagnosed patients of Inflammatory Bowel Disease and forty healthy Controls of both gender ranging in age from 19-50 years were included in the study. Fasting blood samples were processed for following biochemical parameters– Serum Calcium, Phosphorus, Vitamin D, Parathyroid Hormone and Zinc. The subjects were evaluated for Bone Mineral Density (g/cm2) using Dual Energy X-rayAbsorptiometryscan 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. Low Zinc level was observed in 50% of Osteopenic and 80% of Osteoporotic subjects. Zinc level was positively correlated with Bone Mineral Density(r=0.24) and Vitamin D (r=0.25) . Patients with Inflammatory Bowel Disease are more prone to develop metabolic bone disease. Along with other nutrients supplement Zinc should be added to prevent bone loss.

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

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INTRODUCTION

Inflammatory Bowel Disease (IBD) encompasses a heterogeneous group of chronic diseases including Crohn's Disease (CD) and Ulcerative Colitis (UC). The disease causes inflammation of the digestive tract which is traditionally found at opposite ends of the disease spectrum.[1] 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.[2] Systemic nature of IBD also causes extra intestinal manifestations including ankylosing spondylitis, pyoderma gangrenosum, erythema nodosum, iritis, uveitis, episcleritis, primary sclerosing cholangitis, venous thromboembolism, avascular 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.[3] The relative risk of fracture in IBD patients has been estimated to be 40% higher than in general population.[4,5] The pathogenesis of Osteoporosis and the mechanisms of bone loss in IBD patients have not been clearly characterized. Bone loss seems to be multifactorial 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.[6] 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.[7] As a nutrient, Zinc may have some role in bone development as it is needed for osteoblastic activity, collagen synthesis and alkaline phosphatase activity.[8] A study has shown a positive relation between Zinc intake and Bone Mineral Content (BMC) in children.[9] There is paucity of data regarding role of Zinc in IBD and its association with bone metabolism for Indian population. The aim of our study was to investigate serum Zinc level in IBD patients and compare 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. Forty two 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. Forty 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. Vitamin D was analysed on ELISA reader (Kit: DRG) and Parathyroid Hormone was done on Automated–Immunoassay Analyser [Immulite 2000 System]. Serum Zinc 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 Xray 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 means (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).[10]

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 with BMD and bone metabolic parameters. P value <0.05 was considered statistically significant.

RESULTS

Table 1: Baseline characteristics of Study Population

Parameters	Controls (n=40) Mean ±SD (range)	IBD (n=42) Mean ±SD (range)	P value
Age (yrs)	38.56±9.56(19-50)	37.72 ± 12.40(19-50)	NS
Male/Female	25/15	24/18	
Glucose(mg/dl)	84.37±10.16(67-113)	86.85±15.06(61-151)	NS
Cholesterol(mg/dl)	163.92±30.86(89-274)	162.02±37.47(69-274)	NS
Urea(mg/dl)	24.94±7.42(16-45)	24.92±6.81(16-45)	NS
Creatinine(mg/dl)	1.19±0.23(0.6-2.0)	1.17±0.21(0.6-2.0)	NS
Total Protein(g/dl)	6.78±0.50(5.2-7.5)	6.67±0.71(4.3-7.5)	NS
Albumin(g/dl)	3.98±0.46(3.2-4.2)	3.88±0.61(2.0-4.7)	NS
SGOT(IU/L)	26.27±6.25(14-36)	35.54±13.79(20-105)	NS
SGPT(IU/L)	34.22±5.16(17-40)	31.78±11.10(16-62)	NS
ALP (IU/L)	147.63±31.10 (105-221)	139.07 ± 88.75 (60-720)	NS

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

Table 2: The levels of SerumZinc,Bone Mineral indexes and Bone metabolic parameters in IBD subjects and Controls

Character	Mean ± SD (range)				P1	P2	P3
	Control	IBD	UC	CD			
Number	40	42	33	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
Calcium (mg/dl)	9.46±0.79 (8.8-11.0)	9.03±0.99 (6.8-11.8)	9.03±0.98 (6.8-11.8)	8.91±1.14 (7.9-11.8)	0.03	0.04	0.02
Phosphorus (mg/dl)	3.35±0.58 (2.1-4.9)	3.42±0.55 (2.1-4.7)	3.43±0.54 (2.1-4.7)	3.3±1.14 (2.4-4.4)	0.57	0.54	0.80
Vitamin D (ng/ml)	70.34±13.99 (38.2-98.9)	50.66±42.56 (2.6-133.6)	51.05±43.44 (2.6-133.6)	45.74±34.12 ()9.7-126.4	0.006	0.009	0.001
PTH (pg/ml)	62.46±34.46 (11-174)	59.00±36.30 (11-174)	59.11±37.12 (11-174)	57.48±24.76 (30-85.5)	0.65	0.68	0.68

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 3: Serum Zinc,Bone Mineral indexes and Bone metabolic parameters 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	40	12	19	11		
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
Calcium (mg/dl)	9.46±0.79 (8.8-11.0)	9.15±0.85 (7.9-11)	8.99±0.97 (7.6-11.8)	8.2±1.18 (6.8-11.6)	5.86	0.001
Phosphorus (mg/dl)	3.35±0.58 (2.1-4.9)	3.41±0.71 (2.1-4.7)	3.40±0.49 (2.4-4.4)	3.36±0.44 (2.6-4.6)	0.05	0.98
Vitamin D (ng/ml)	70.34±13.99 (38.2-98.9)	54.31±43.35 (7.2-133.6)	49.85±43.40 (5.9-132.0)	44.81±41.56 (2.6-133.0)	2.9	0.03
PTH (pg/ml)	62.46±34.46 (11-174)	54.94±31.22 (11-105)	58.17±30.70 (11-122)	67.35±48.38 (12-174)	0.2	0.82

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).

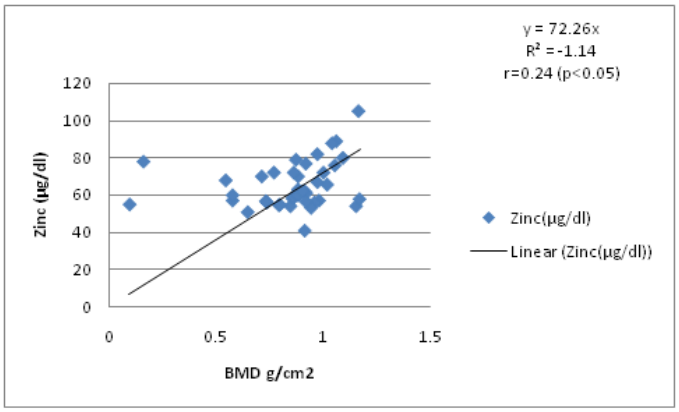

Figure 1: Correlation between Zinc and BMD

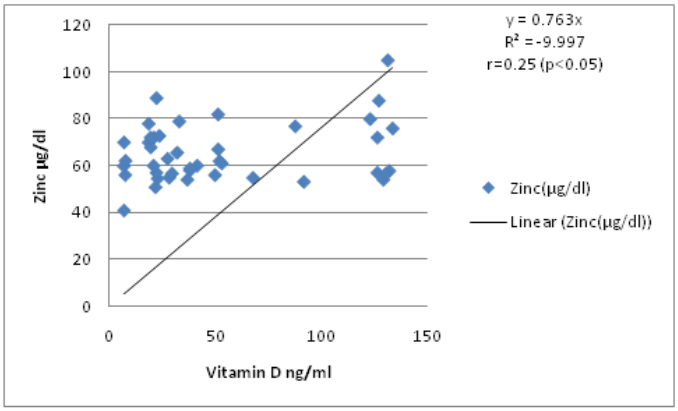

Figure 2: Correlation between Copper and BMD

The mean age of the 42 IBD patients was 37.72 ± 12.40 years at the time of diagnosis. No patient had family history of IBD. Table 1 shows the baseline biochemical findings. No significant difference in routine parameters i.e. Sugar, Urea, Creatinine, Total Protein, Albumin, AST, ALT and ALP was observed between IBD and Controls. 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 2). The mean lumbar spine BMD values and serum Zinc level of the patients with IBD was significantly low as compared with the Controls (p<0.001).The mean lumbar spine BMD and serum Zinc levels of the Cronhs Disease and Ulcerative Colitis groups were also significantly low as compared to Control group (p<0.05). On evaluating the bone metabolic parameters in subjects, the mean serum Calcium level of IBD, CD and UC groups were significantly lower than Control group (p<0.05). Serum Vitamin D levels of IBD, UC and CD groups were 50.66±42.56, 51.05±43.44 and 45.74±34.12 ng/ml. Vitamin D showed statistically significant mean level difference between IBD and Controls, Controls and UC, and Controls and CD groups (p<0.005). However no significant difference in serum PTH and Phosphorus levels could be observed between Control and IBD, CD and UC groups. 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 (10). Among 42 IBD patients we observed that 28.5% IBD patients had Normal bone density, 45.2% were Osteopenic and 26.2% had Osteoporosis (Table 3).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(71.4)% .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 was lowest in Osteoporotic group and mean level difference of Zinc among all the groups were highly 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 levels (p<0.0001). Serum Calcium was observed significantly low in Osteoporotic group, the mean value was 8.2±1.18 mg/dl while in Osteopenic group of IBD patients mean of Calcium was found 8.99±0.97 mg/dl. Further using ANOVA the mean difference of Calcium was highly statistically significant among all the groups (p=0.001). However no significant mean difference was found in Phosphorus among all thegroups. Vitamin D was least in Osteoporotic group and on using ANOVA the mean difference was statistically significant among all the groups. However PTH showed no significant change among all the groups. Serum Zinc was significantly positively correlation with BMD (r=0.24,Fig 1) and Vitamin D (r=0.25,Fig 2).

DISCUSSION

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.[11, 12] Fragility fractures, the hallmark of Osteoporosis, are a major cause of morbidity and mortality. Osteoporosis risk is also increased in a number of diseases with an inflammatory component, such as Inflammatory Bowel Disease.[13,14] Nutrients and lifestyle plays an important

role in bone health. Patients with IBD reveal various malnutrition statusand in many studies deficiency of trace elements have been reported as characteristics feature of malnutrition.[15] Underlying mechanism postulated as causing Mineral deficiency in IBD include inadequate dietary intake decreased absorption, increased requirements and increased losses.[16] 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.[17] 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.[18] 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.[19] Bone is highly dynamic and it undergoes constant remodelling throughout life. The remodelling involves coupled resorption of existing bone and the formation of new bone. The supply of Calcium and inorganic Phosphate available for bone formation is a result of highly regulated and complex homeostatic mechanism involving Para cellular and trans cellular intestinal and renal reabsorption.[20] Vitamin D plays a key role in skeletal and Mineral homeostasis. It is frequently viewed as a pro-bone anabolic hormone because of its positive effects on intestinal and renal Calcium and Phosphorus reabsorption and through its positive effects on osteoblast differentiation and bone matrix synthesis.[21] The aim of our study was to assess the relationship between the serum Zinc level and the bone Mineral indexes. 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 level than UC patients, but the difference was insignificant. Further we observed that along with the Zinc other macronutrients like Calcium, Phosphorus and fat soluble Vitamin like Vitamin D were also significantly low in IBD patients ascompared toControls. All these nutrientsseem to reduce the BMD in IBD patients. Metabolic bone disease develops silently in these patients, the origin of which is probably multifactorial: lack of physical activity, deficiencies of Calcium, Phosphorus, Vitamins and other micronutrients (Zinc). Intestinal malabsorption has been intuitively linked to the pathogenesis of bone loss in IBD and celiac disease patients.[22] 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 low serum Zinc level while in Osteoporotic group 80% patients had low serum Zinc level. Further serum Zinc level was positively correlated with Bone Mineral Density (r=0.24) and Vitamin D (r=0.25) in Inflammatory Bowel Disease patients. Thus correction of zinc 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.Nutrition plays an important role in the management ofpatients with IBD. To minimize risks of metabolic bone disease, patients with IBD should be given Zinc supplements along with other nutrients to prevent bone loss.

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REFERENCES

1. Natalie A. Increasing Incidence and Prevalence of the Inflammatory Bowel Diseases With Time, Based on Systematic Review. Gastroenterology. 2012; 142:46–54.
2. Shikhare G, Kugathasan S. Inflammatory Bowel Disease in children: current trends. J Gastroenterol. 2010; 45: 673–682.
3. Larsen S, Bendtzen K , Nielsen OH. Extra intestinal manifestations of Inflammatory Bowel Disease: epidemiology, diagnosis, and management. Ann Med. 2010; 42: 97–114.
4. Ali T, Lam D, Bronze MS, Humphrey MB. Osteoporosis in Inflammatory Bowel Disease. Am J Med. 2009; 122: 599–604.
5. Amber J, Tresca V. Osteoporosis in Inflammatory Bowel Disease. Am J Med . 2009; 122: 599–604.
6. Tania M .The nutriceutical bovine colostrum truncates the increase in gut permeability caused by heavy exercise in athletes. Am J Physiol Gastrointestinal Liver Physiol.2011; 300: G477–G484.
7. Prentice A, Schoenmakers I, Laskey MA. Nutrition and bone growth and development. Proc Nutr Soc. 2005; 65:348- 360.

8. Palacios C. The role of nutrients in bone health, from A to Z. Crit Rev Food Sci Nutr. 2006; 46:621-8.
9. Bounds W, Skinner J, Carruth BR, Ziegler P. The relationship of dietary and lifestyle factors to bone Mineral indexes in children. J Am Diet Assoc. 2005; 105:735-41.
10. WHO. Assessment of fracture risk and its application to screening for postmenopausal Osteoporosis. Report of a WHO Study Group. World Health Organ Tech Rep Ser. 1994; 843:1-129.
11. European Commission Report on Osteoporosis in the European Community: Action for Prevention. Luxembourg: Office for Official Publications for the European Commission.1998.
12. International Osteoporosis Foundation (IOF): Facts and statistics about osteoporosis and its impact in 2007. Available at: www.iofbonehealth.org/facts-and-statistics.html. Accessed September 19, 2007.
13. Scott EM, Gaywood I, Scott BB. Guidelines for osteoporosis in coeliac disease and inflammatory bowel disease. British Society of Gastroenterology. Gut. 2000; 46:1–8.
14. Bernstein CN, Leslie WD. Osteoporosis and inflammatory bowel disease. Aliment Pharmacol Ther. 2004; 19:941–52.
15. Alfredo J, Lucendo LC, Rezende DE. Importance of nutrition in Inflammatory Bowel Disease. World J Gastroenterol. 2009 May 7; 15(17): 2081-2088.
16. Fernández-BF, Mingorance MD. Serum zinc, copper, and selenium levels in inflammatory bowel disease: effect of total enteral nutrition on trace element status. American Journal of Gastroenterology. 1990; 85 (12): 1584-1589.
17. Rezvan R, Ensieh E. Association of Zinc, Copper and Magnesium with bone Mineral density in Iranian postmenopausal women – a case control study. Journal of Diabetes and Metabolic Disorders. 2014, 13:43-48.
18. Caleb O, Molokwu BS, Yang V, Li MB. Zinc Homeostasis and Bone Mineral Density .Ohio Research and Clinical Review.www.oucom.ohiou.edu/dbms-li/orcr-LiandMoloku.pdf .Fall 2006; 15.
19. Griffin IJ, Kim SC, Hicks PD, Liang LK, Abrams SA. Zinc metabolism in adolescents with Crohn's disease. Pediatr Res. 2004 ;56(2):235-9.
20. Bronner F. Recent developments in intestinal calcium absorption .Nutr Rev .2009; 67:109-113.
21. Maitreyi R. Vitamin D and gastrointestinal diseases: inflammatory bowel disease and colorectal cancer. Therap Adv Gastroenterol.2011;4(1):49-62.
22. Corazza GR, Di Stefano M, Maurino E, Bai JC .Bones in coeliac disease: diagnosis and treatment. Best Pract Res Clin Gastroenterol.2005; 19: 453–465.

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