DOI Number: 10.37506/v14/i1/2020/ijfmt/193030

Risk of Osteoporosis in Patients with Crohn's Disease Not Respond to Infliximab Therapy Undergoing Surgical Intervention

Fatima A.Mohammad[1], Fatin Fadhel ALkazazz[2], Sura A.Abdusattar[3]

[1]Department of Chemistry, College of Science, Mosul University, Iraq, [2]Department of Chemistry, College of Science, AL Mustasiyriah University, Iraq, [3]Chemistry and Biochemistry Department, College of Medicine, AL Mustasiyriah University, Iraq

Abstract

Osteoporosis is one of the most common complications of Crohn's disease (CD). Proper history with blood samples were collected from (30) healthy control group, (30) Crohn's disease patients have been respond to biological therapy (infliximabIFX) (response group) and (30) CD patients with (non-response group) to biological therapy undergoing surgical intervention for the estimation of some biochemical parameters. This study demonstrate a significant decrease in FGF-23 (fibroblast growth factor 23) and vitamin D Levels between (non- response group) and control group ($p< 0.01$). Similary , parathyroid hormone (PTH) Levels were increased significantly ($p< 0.01$) in (non- response) group. Moreover the calcium (Ca) and phosphours (P) decreased significantly ($p< 0.01$) in same group. While in CD patients that have been treated with infliximab only vitamin D and FGF 23 decreased significantly ($p< 0.01$)compared with control group. Levels of Serum FGF23 are considerably lower in CD patients, It is independent pattern was not affected by type of treatment (medical or surgical) . Hypovitaminosis common finding in CD patient's .Although CD is not disease of vit D deficiency but it clearly is a disease whose pathogenesis seems closely related to vit D Level.

Keyword: *Osteoporosis, Crohn's disease, Infliximab, Vitamin D*

Introduction

Inflammatory bowel disease (IBD) includes two lasting intestinal disorders, which are: CD and Ulcerative colitis (UC) [1] CD is a chronic inflammatory disorder that could involve any part of alimentary tract from mouth to anus [2]. These disorders were first described by Dr. Burril Crohn's and his team in 1932 [3]. Although its aetiopathogenesis is still not clear, it has been well recognized that CD is one of the complicated disorders which result from interaction of environmental, microbial, and genetic factors[4] .Patient that have Crohn's disease are under increased risks of the metabolic bone disease, which includes osteoporosis and osteopenia [5] . They seem to be one of the most important complication since osteoporosis has been reported in (30%-50%) of patient with CD which may be a silent disease until a fracture occurs [6] . BMD (Bone mineral density) is less in patients that have CD [7] . The mechanism which is underlying the lower BMD hasn't been clearly understood [8] . None-the-less, various risk factor including increasing age [9] disturbances of calcium homeostasis, malabsorption and vitamin D, smoking and the actual inflammatory process by releasing cytokines that interact with bone metabolism[10]. Malabsorption and the deficiency of vitamin D which results from the involvement of the small intestinal of disease and or surgical resections are usually found in patients that have CD [11]. Which is why, the deficiency of vitamin D may result in hypocalcemia in patients that have Crohn's disease with short bowel syndrome, also hypocalcemia might be resulting from deficient parathyroid hormone which stimulates bone resorption resulting in bone loss [12] . On the other hand, regulation of the phosphate in bone homeostasis hasn't been entirely explained, regulation of the phosphate includes interaction of a complicated kidney–intestine–bone parathyroid gland hormonal axis that remains insufficiently comprehended. A lately discovered phosphatonia, FGF23 disclosed now. Pathways in mineral metabolism pathophysiology [13] . FGF23 is a new phosphaturic hormone which is

mainly generated by osteoblasts/osteocytes in bones, and with lower levels in other types of tissue, they target the kidneys for regulating vitamin D metabolism and phosphate homeostasis [14]. Infliximab anti-body directed against TNF (tumor necrosis factor alpha), has been the first TNF antagonist which has been approved for patient that have CD [15]. It was an important advance in the treatment of the disease due to their proven efficacy in inducing and maintaining clinical symptoms remission [16]. 74% of all CD patients will require surgery[17]. Surgical management typically with intestinal resection remains considered as a significant modality of treatment because of disease symptoms nature and complication namely strictures and perforation obstruction, starts so hard to be sufficiently controlled [18] .

Aim of study : Aim of this study are characterize serum PTH, Ca, P, vit D , and FGF 23 levels at Crohn's disease patient's compared with controls , and to identify correlates of changes in these parameters levels following therapy.

Material and Methods:-

Study subject

This research has been approved by the Ethics committee, department of chemistry, college of science , Mustansiriyah University , Bagdad , Iraq. and the Iraqi Ministry of Health approved this work as well. The blood samples were taken after informed consent of participant were recruited from Gastroenterolology and Hepatology teaching hospital at Bagdad Medical city, while the healthy group were volunteers. All the patients were diagnosed by senior doctors specialist in gastroenterology field, (60) sixty unrelated Iraqi Crohn's disease patient's divided in to two groups according to response to biological therapy (infliximab) the first group (30) patients was respond to infliximab according to classical regimen (loading dose 5mg/kg at week 0,2,and 6 followed by repeated infusion of 5mg/kg every 8 weeks) and (30) patients not respond to infliximab undergoing surgical intervention as well as (30) unrelated healthy person termed as control group without any systemic disease. All the patients and control aged between 18 and 64 years. As well as the two groups of patient's and control were measured by electronic balance and measuring body mass index (BMI). Five milliliters of venous blood was obtained from patients and control group by 5 ml disposable syringe (without tourniquet) drained into get plain tubes and left in room temperature (25C°) for 15 minutes, Then it was centrifuged at 2000 xg for 10 minutes in order to collect sera. Sera aliquots were placed in eppendorf tubes and stored at -40C° until used.

Biochemical analysis

The human FGF-23- was measured in the sera for the all patient and control group using the double sandwich (ELISA) kit according to manufactures instruction (Cat No.MBS 263043, My bio source / USA). PTH was measured using the protocol of ELISA kit (Cat No.MBS2505074,My bio source /USA). And vitamin D was measured using the competitive (ELISA) kit principle (Cat No.MBS 2503525 , My bio source / USA). While Calcium and phosphorus determined by automatically performed by the Dimension ® Clinical chemistry system (SIEMENS, Germany).

Statistical analysis

The statistical analysis system SAS [19] program has been utilized to compare between control and two CD patients groups (response and non-response to biological therapy) in study parameters. (Analysis of variation-ANOVA) was used to compare between means (P value of 0.05 and 0.01 has been considered to be statistically significant).

Result and Discussion

Mean ± SD value of age and BMI were recorded from all subscribers as shown in table 1. Results of this study shown in table 2 there were a non-significant differences in mean age between all age of the studied group at ($p<0.05$). Age at the onset of CD was in the range between early childhood to beyond 70 years of age [20]. Although the peak incidence of diseases between ages 20 to 39, about 25% of patients with Crohn's is present before 20 years of age–among children with Crohn's disease, 4% present before 5 years of age [21]. a second peak is recognized between ages 50-70 [22].Age definitions of early and elderly onset of Crohn's in literature are different and are usually dependent on local clinical practice (such as age of referring from pediatric to adult Crohn's disease care), and that makes comparisons between population complicated. In addition to that, heterogeneity in the approaches of data collection and case ascertainment (such as, diagnostic criteria, access to diagnostic procedures) – and as a result, differences in the capability of capturing each case of Crohn's disease in the population- might result in bios in any of those

comparisons [23] also shown that CD patients have a significant BMI decrease (P<0.01) from (26.743±2.699) Kg/m^2 to (22.816±2.025) Kg/m^2 in (non-response group) patients need surgical intervention compared with control healthy group this could be represented with malabsorption, decreased dietary intake, metabolic disturbances like increased expenditure of energy, increased substrate oxidation rates, and decreased respiratory quotient, could as well be one of the reasons that cause patient's protein, glucose, and fat loss. To some extent, medical theropy including corticosteroid, mesalamine, TNF- alpha antagonists, and azathioprine, could enhance the BMI of CD patients [24].

The laboratory examination data in the three groups are shown in table(2),also table (3) illustrated the statistics comparison of different biochemical analysis among the studied groups. Serum parathyroid hormone, calcium and phosphate were not significantly charged in patient response to infliximab group compared with healthy control group. The FGF23 and vitamin D significantly decreased (P>0.01) between the same groups. A research in the animal model TNF- mediated bone erosion have showed that treating mice with anti-TNF factor combined with osteoprotegerin or PTH results in repairing local bone erosions[25].There for the treatment with infliximab [26]or adalimumab have an advantageous impact on bone metabolism in patients that have CD [27].

Abreu, etal. [26] have reported that patients that have Crohn's disease have appropriately increased Levels of active hormonal form of vitamin D 1,25 $(OH)_2D$. Those Level are inversely correlated to BMD probably due to the fact that 1,25 $(OH)_2D$ mobilizes the stores of skeletal calcium, the mechanism for increasing 1,25$(OH)_2$ D is via increased 1-α hydroxylase expression by activated lamina propria macrophage, that some other way in which IFX has a positive impact on bone metabolism is via inhibiting macrophage activation in gut and decreased expression of 1,α-hydroxylase on the other hand vitamin D is essential for absorbing calcium in small bowel over time, vitamin D deficient generates hypocalcemia and consequently a secondary increase in the PTH Level [28] also the regulation of calcium and phosphate metabolism, as physiological correlation between the levels of its main regulators PTH and vitamin D [29].

While there is significantly increase (p>0.01) in PTH levels and significantly decrease (p>0.01) in Levels of Ca, P and vitamin D in(non-response group) patients that surgical treated compared to control group in this study parathyroid hormone is released from parathyroid cells as a response to low extra cellular concentration of free calcium. Secondary hyperparathyroidism has been detected in more than 1/3 of patients that have Crohn's disease who have operated on with small bowel resection, increased levels of PTH were related to increased bone turnover and decreased BMD preferentially in cortical bone [11] .

Table 1. Statistical analysis of Age and BMI, measurements distributed among patients of Crohn's disease (response and non-response) and control groups.

	Group	Mean ± SD	SE	95% C.I. for Mean		Min.	Max.
				L.b.	U.b.		
Age (Years)	A	40.551±10.715	1.989	36.475	44.627	19.00	55.00
	B	37.700±11.262	2.056	33.494	41.905	18.00	64.00
	C	38.500±9.846	1.797	33.823	41.176	18.00	5800
BMI (Kg/m^2)	A	26.743±2.699	0.5012	25.917	27.970	22.91	32.81
	B	25.590±2.063	0.476	25.020	24.561	21.74	30.11
	C	22.816±2.025	0.369	22.059	21.572	17.20	23.70

Group A: control groups without any treatment, Group B: patients of Crohn's disease responded to infliximab therapy ((response group) , Group C: group patients of Crohn's disease undergoing surgical intervention (non-response) group , SD: Standard deviation, SE: Standard error, 95% C.I.: 95% Confidence interval, L.b.: Lower Bound, U.b.: Upper Bound, Min: minimum and Mix: Maximum.

Table 2. Statistical analysis of PTH, Ca, P, FGF 23 , and VitD parameters distributed among patients of Crohn's disease (response and non-response) groups and control group.

Parameter	Group	Mean±SD	SE	95% C.I. for Mean		Min.	Max.
				L.b.	U.b.		
PTH (pg/ml	A	84.892±11.613	1.156	79.074	87.909	59.41	103.50
	B	86.182±8.337	1.522	83.069	89.295	67.82	111.42
	C	123.724±37.926	6.924	109.561	137.886	79.31	216.42
Ca (mg/dL	A	8.133±0.709	0.131	7.863	8.403	6.56	9.20
	B	7.663±0.641	0.117	7.423	7.902	6.73	9.84
	C	6.568±2.021	0.369	4.813	6.323	2.31	8.71
P (mg/dL	A	3.648±0.695	0.129	2.984	3.513	2.01	4.40
	B	3.521±0.523	0.095	3.325	3.716	2.60	4.90
	C	2.185±0.875	0.159	1.858	2.512	0.84	4.10
FGF (pg/ml	A	1047.189±27.113	5.034	1036.876	1057.503	1006.20	1094.40
	B	733.605±54.117	12.801	747.423	799.788	640.31	932.40
	C	744.064±50.337	9.190	725.267	762.860	680.52	830.30
VitD (pg/ml	A	29.983±12.677	2.354	25.161	34.805	4.61	55.40
	B	2.187±1.047	0.191	1.796	2.578	0.71	4.82
	C	1.1169±0.994	0.181	0.797	1.540	0.01	4.82

Group A: control groups without any treatment, Group B: patients of Crohn's disease treated with infliximab (response group), Group C: patients of Crohn's disease undergoing surgical intervention (non-response) group, SD: Standard deviation, SE: Standard error, 95% C.I.: 95% Confidence interval, L.b.: Lower Bound, U.b.: Upper Bound, Min: minimum and Mix: Maximum.

Table 3: Multiple comparison significant (ANOVA) for parameter between the different groups.

Groups	**PTH (pg/ml)**	**Ca (mg/dL)**	**P (mg/dL)**	**FGF (pg/ml)**	**VitD (pg/ml)**
	P-Value				
A & C	0.0012 **	0.001 **	0.001 **	0.001 **	0.001 **
B & C	0.002 **	0.002 **	0.003 *	NS	NS
A & B	NS	NS	NS	0.002 **	0.001 **

Conclusion

The conclusions that have been drawn in this paper have stated the fact that Levels of serum FGF23 are considerably lower in patients that have Crohn's disease, it is an independent pattern not affected by type of treatment (medical or surgical). Also hypovitaminosis D was common finding in CD patients Although Crohn's is not a condition of the deficiency of vitamin D but it

is clearly a disease whose pathogensis seems closely related to the Level of vitamin D. in addition to that, patients that have CD subjected to surgical intervention are under a risk to develop low Bone mineral density and secondary hyperparathyroidism.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Science, Mosul University, Iraq and all experiments were carried out in accordance with approved guidelines.

References

1. Su H-J, Chiu Y-T, Chiu C-T, Lin Y-C, Wang C-Y, Hsieh J-Y, et al. Inflammatory bowel disease and its treatment in 2018: Global and Taiwanese status updates. J Formos Med Assoc.xx,le10.
2. Feldman M, Friedman LS, Brandt LJ. Sleisenger and fordtran's gastrointestinal and liver disease E-Book: pathophysiology, diagnosis, management, expert consult premium edition-enhanced online features. Vol. 1. Elsevier Health Sciences. 2016.
3. Fatahi DA, Al Asmari AS, Bukhari GA, Alshamrani HA, Hunaydi KA, Sharahili AM, et al. Crohn's Disease: Pathophysiology, and Management. Egypt J Hosp Med (January 2018). 2018;70(11):2004–7.
4. McGovern DPB, Kugathasan S, Cho JH. Genetics of inflammatory bowel diseases. Gastroenterology. 2015;149(5):1163–76.
5. Podolsky DK, Camilleri M, Fitz JG, Kalloo AN, Shanahan F, Wang TC. Yamada's textbook of gastroenterology. John Wiley & Sons. 2016; 1423.
6. Thodis E, Rossos P, Habal F, Oreopoulos D. Negative impact of Crohn's disease on bone mineral mass. J Musculoskelet NEURONAL Interact. 2003;3(3):246–50.
7. Habtezion A, Silverberg MS, Parkes R, Mikolainis S, Steinhart AH. Risk factors for low bone density in Crohn's disease. Inflamm Bowel Dis. 2002;8(2):87–92.
8. Schoon EJ, Geerling BG, Van Dooren IMA, Schurgers LJ, Vermeer C, Brummer R, et al. Abnormal bone turnover in long-standing Crohn's disease in remission. Aliment Pharmacol Ther. 2001;15(6):783–92.
9. Siffledeen JS, Siminoski K, Steinhart H, Greenberg G, Fedorak RN. The frequency of vitamin D deficiency in adults with Crohn's disease. Can J Gastroenterol Hepatol. 2003;17(8):473–8.
10. Tilg H, Moschen AR, Kaser A, Pines A, Dotan I. Gut, inflammation and osteoporosis: basic and clinical concepts. Gut. 2008;57(5):684–94.
11. Jahnsen J, Falch JA, Mowinckel P, Aadland E. Vitamin D status, parathyroid hormone and bone mineral density in patients with inflammatory bowel disease. Scand J Gastroenterol. 2002;37(2):192–9.
12. Fukumoto S, Namba N, Ozono K, Yamauchi M, Sugimoto T, Michigami T, et al. Causes and differential diagnosis of hypocalcemia-recommendation proposed by expert panel supported by ministry of health, labour and welfare, Japan. Endocr J. 2008;804240102.
13. Oikonomou KA, Orfanidou TI, Vlychou MK, Kapsoritakis AN, Tsezou A, Malizos KN, et al. Lower fibroblast growth factor 23 levels in young adults with Crohn disease as a possible secondary compensatory effect on the disturbance of bone and mineral metabolism. J Clin Densitom. 2014;17(1):177–84.
14. El-Hodhod MA-A, Hamdy AM, Abbas AA, Moftah SG, Ramadan AAM. Fibroblast growth factor 23 contributes to diminished bone mineral density in childhood inflammatory bowel disease. BMC Gastroenterol. 2012;12(1):44.
15. Billiet T, Cleynen I, Ballet V, Ferrante M, Van Assche G, Gils A, et al. Prognostic factors for long-term infliximab treatment in Crohn's disease patients: a 20-year single centre experience. Aliment Pharmacol Ther. 2016;44(7):673–83.
16. Taxonera C, Robledo P, Rodríguez A. Treatment persistence during therapeutic sequences with adalimumab and infliximab in the treatment of Crohn's disease. Rev Española Enfermedades Dig. 2017;109(10):690–3.
17. Prasad MA, Friedman LS. Peptic Ulcer Disease. Sitaraman Friedman's Essentials Gastroenterol. 2017;126.
18. Saeed M, Hullur H, Salem A, Ali A, Sahib Y, Ashfaq M. Improved Biochemical Parameters in Patients Who Undergo Early Resection in Isolated Ileocaecal Crohn's Disease. Surg Res Pract. 2017;2017.

19. Stokes ME, Davis CS, Koch GG. Categorical data analysis using SAS. SAS institute; 2012.
20. Arnott I, Rogler G, Halfvarson J. The Management of Inflammatory Bowel Disease in Elderly: Current Evidence and Future Perspectives. Inflamm Intest Dis. 2017;2(4):189–99.
21. Rosen MJ, Dhawan A, Saeed SA. Inflammatory bowel disease in children and adolescents. JAMA Pediatr. 2015;169(11):1053–60.
22. Nimmons D, Limdi JK. Elderly patients and inflammatory bowel disease. World J Gastrointest Pharmacol Ther. 2016;7(1):51.
23. Duricova D, Burisch J, Jess T, Gower-Rousseau C, Lakatos PL, ECCO-EpiCom. Age-related differences in presentation and course of inflammatory bowel disease: an update on the population-based literature. J Crohn's Colitis. 2014;8(11):1351–61.