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RESEARCH ARTICLE

Bone Mineral Density and Bone Remodeling in Tunisian Patients with Inflammatory Bowel Disease

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Abstract:

Background:

A high prevalence of osteopenia and osteoporosis is observed in patients with Inflammatory Bowel Disease (IBD).

Objective:

The aim of our study was to investigate the prevalence of bone loss, bone remodeling and risk factors in Tunisian patient with IBD.

Patients and Methods:

The study included 40 patients with IBD and 32 age- and sex-matched healthy controls subjects. All participants underwent bone densitometry by dual energy X-ray absorptiometry at the femoral neck and lumbar spine. Serum levels of 25-hydroxy vitamin D (25(OH)D), parathyroid hormone (PTH), osteocalcin(OC), and urinary degradation products of C-terminal telopeptide of type I collagen (CTXI) were measured in all participants to assess the bone metabolism status.

Results:

Twelve (30%) patients were normal, 32.5% were osteopenic and 37.5% were osteoporotic. Osteoporosis was more frequent in IBD patients than controls (p=0.0001). Age and inflammation were associated with low bone mineral density (BMD). Mean calcium, phosphorus and alkaline phosphatase levels were similar in both groups. Median 25(OH) D levels were significantly lower in IBD patients compared with controls (p=0.0001). Median urinary CTXI levels were significantly higher in IBD patients compared with healthy controls (p=0.007). No significant differences between IBD patients and controls concerning the median serum OC and PTH levels were found.

Conclusion:

In our study, there is a high prevalence of low BMD in IBD patients and an increase in bone resorption without a change of bone formation. Low BMI and hypovitaminoses D were identified as risk factors for low BMD.

Keywords: Inflammatory bowel disease, Osteopenia, Osteoporosis, Hypovitaminosis D, Bone mineral density, Bone remodeling.

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1. INTRODUCTION

There is a growing body of evidence that patients with Inflammatory Bowel Disease (IBD) are at increased risk for osteopenia and osteoporosis. Osteopenia and osteoporosis are common extraintestinal complications in IBD that increase the risk of vertebral and femoral neck fractures [1, 2].

The prevalence of osteoporosis is changeable depending on the studied population and the technique of bone density measurement used [3]. Certain factors are related to the disease itself (intestinal inflammation, extent of lesions, disease duration), whereas others depend on the patient himself (age, body mass index, nutritional status, hormonal status) or the treatment (corticosteroids or surgical resection) [1, 2].

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Several studies have demonstrated a decrease in Bone Mineral Density (BMD) in patients with Crohn's Disease (CD), and to a less extent in Ulcerative Colitis (UC) [4]. The higher prevalence of bone disease in CD patients is thought to be related to ileal and small intestine involvement of disease-causing vitamin D and calcium malabsorption, estrogen deficiency and malnutrition [1, 5]. However, some recent studies have found no significant differences in the prevalence of osteoporosis between patients with UC and those with CD [6, 7].

Bone tissue is constantly being renewed and repaired by a coupled process of resorption and formation called bone remodeling. In this study, we aimed to estimate the prevalence of bone loss and the rate of bone remodeling in Tunisian adults patients with IBD, and identify the potential risk factors of bone loss.

2. PATIENTS AND METHODS

2.1. Patients

This is a single-centre clinical cross-sectional and comparative study, conducted between October 2013 and June 2014. In this period, forty IBD patients who followed up at the Department of Gastroenterology and Rheumatology at Hedi Chaker University Hospital were included in this study. Our study received research ethics board approval from the "C.P.P. SUD" (0069/2018) committee. Thirty two age- and sex-matched healthy subjects were selected as controls. All participants, patients and controls, were of Tunisian origin.

The exclusion criteria of the study included diseases known to affect bone metabolism such as malignant diseases, celiac disease, short bowel disease, kidney failure, liver disease, thyroid disease, diabetes mellitus and the use of medications such as bisphosphonates, sodium fluoride, calcitonin and hormone substitution therapy.

The diagnosis of UC and CD was based on standard criteria including clinical criteria, endoscopy and histopathological biopsy [8, 9] and the Montreal classification was used for disease phenotyping [10]. The activity and severity of diseases were evaluated based on the Crohn's disease activity index (CDAI) for CD and the Truelove-Witts score for UC [11, 12].

For each patient, the following data were collected: age, gender, menopausal status for females, smoking status, physical activity, solar index (number of hours of sun exposition X exposed part of the body (%)), body mass index (BMI) in Kg/m2, location, extent and duration of the disease, ongoing treatments, history of surgical resection, cumulative corticosteroid dose (expressed in g prednisone equivalent) and duration of corticosteroid treatment. Informed consent was obtained from all participants.

2.2. Methods

2.2.1. Biochemical Markers

Blood and urine samples were collected from all IBD patients and control subjects in the morning after an overnight fast. Then, they were stored at -80°C until assayed. Calcium, phosphorus, total serum alkaline phosphate and creatinine were determined with an optimized method using a CX9 Beckmann Coulter automate. Urinary CrossLaps were measured with an immunoenzymatic method.

Serum intact parathyroid hormone (PTH), 25 hydroxy vitamin D (25(OH) D) and serum osteocalcin were measured by an electro-chemiluminescence immunoassay ECLIA (Roche Elecsys2010).

Laboratory parameters such as albumin and Erythrocyte Sedimentation Rate (ESR) were also checked in all patients.

The serum calcium was corrected with albumin level using this formula:

Corrected calcium level = measured calcium level + 0,025 x (40-albumin).

Table **1** shows the biochemical markers which were measured in IBD patients and controls, their analytical method, their normal range and their unit.

Table 1. Biochemical markers measured in Inflammatory Bowel Disease patients and controls, their analytical Method, their normal range and their unit.

Biochemical Markers	Analytical Method	Normal Range	Unit
Calcium	Colorimetric Arsenazo [13]	2,2 - 2,6 [13]	mmol/l
Phosphorus	Colorimetric phosphomolybdate complex [14]	0,5 - 2 [14]	mmol/l
Alcaline phosphatase	Kinetic rate method [15]	38 - 126 [15]	UI/L
Creatinine	Jaffe method [16]	Male (70-120) Female (60-110) [16]	µmol/l
Erythrocyte sedimentation rate	Wintrobe method	Male = age/2 Female = age + 10/2	mm
25-OH Vitamin D	Electro-chimiluminescence immunoassy ECLIA [17]	≥ 30 [17 - 20]	ng/ml
Parathyroid hormone	Electro-chimiluminescence immunoassy ECLIA [21]	15 - 65 [21]	pg/ml

(Table 1) contd.....

Biochemical Markers	Analytical Method	Normal Range	Unit
Serum osteocalcin	Electro-chimiluminescence immunoassy ECLIA [22]	Female: Premenopausal female: 11 - 43 Menopausal female:15 - 46 Osteoporotic: 13 - 48 Male: 18 à < 30 years: 24 à 70 30 à 50 years: 14 à 42 > 50 à 70 years: 14 à 46 [22]	ng/ml
Urinary Cross Laps	ELISA immunoassay [23]	Menopausal female: 67-544 Premenopausal female: 121-874 Male: 54-559 [23]	µg/mmol urinary creatinine
Albumin	Colorimetric bichromatic digital endpoint methodology [24]	34-48 [24]	g/l

Table 2. Study population demographic and clinical parameters.

Parameters	IBD Group	Control Group	p
Number N	40	32	
Mean age (years)	43.53 ± 13.96 (16-71)	44.72 ± 14.12 (16-70)	0.721 ¶
Sex (%) Female Male	23(57.5%) 17(42.5%)	15(46.8%) 17(53.2%)	0.255 ¥
Current smokers (%)	12(30%)	7(22%)	0.3 ¥
Mean Solar Index	0.59± 0,16 (0.31-0.71)	0.61 ± 0,25 (0.31-1.26)	0.628 ¶
Physical activity (%) Moderate activity Sedentariness	23(57.5%) 17(42.5%)	28(87.5%) 4(12.5%)	0.23 ¥
Menopausal women (%)	14(34.8%)	13(40%)	0.65 ¥
Duration of menopause(years)	7.75± 6.8 (1-20)	9.6± 6.8 (2-18)	0.59¶
Mean BMI (kg/m^2)	20.98 ±3.59 (13,84 - 32,81)	25.05 ± 3.96 (17,11 - 32,41)	0.001 ¶

¶ Student - test; ¥ Chi-squared test. IBD: Inflammatory Bowel Disease; BMI: Body Mass Index

2.2.2. Bone Mineral Density Measurements

BMD was evaluated at the lumbar spine (L1 to L4) and the femoral neck using the Lunar Prodigy Dual-energy X-ray Absorptiometry (DXA) system.

BMD results were expressed as T-scores which is the number of Standard Deviations (SD) of the patients BMD from the mean peak value for a reference population with the same sex and race. Patients were classified according to the World Health Organisation (WHO) criteria [25] as normal (T-score > -1 SD at both lumbar spine and femoral neck), osteopenic (T-score from -1.0 SD to -2.5 SD at either lumbar spine or femoral neck or both) and osteoporotic (T-score ≤ -2.5 at either lumbar spine or femoral neck or both).

2.2.3. Statistical Analysis

Statistical analysis was carried out using the Statistical Package for Social Science (SPSS) program for Windows® version 20.0 software. Data were presented as the number (%) and mean ± Standard Deviation (SD) or median (range: min – max). Differences between groups were compared by Student test for continuous variables or Mann-Whitney test (nonparametric test) and by Chi-squared test for categorical variables. Correlations between continuous variables were assessed using Spearman rank correlation. A p value <0.05 was considered statistically significant.

All authors had access to the study data and reviewed and approved the final manuscript.

3. RESULTS

The main characteristics of the study subjects are shown in Table **2**.

In all, 40 Tunisian IBD patients and 32 age- and sex-matched healthy controls were enrolled in the study. No participants had a History of drug use related to bone density such as bisphosphonate, vitamin D 3, SERM, denosumab and statin.

Nineteen (47.5%) patients were identified as having CD and 21 (52.5%) patients as having UC.

There were 17 men and 23 women in the total group. In patients with CD, male to female ratio was 0.72 and in UC patients, it was 0.75. Mean age was 43.53 ± 13.96 years (range 16 – 71) in all IBD patients whereas it was 45.14 ± 13.52 years (range 16 – 71) and 41.74 ± 14.59 years (range 19 – 62) in UC and CD patients, respectively.

There was no statistically significant difference in age, sex, smoking, solar index, physical activity and menopausal status between the IBD and the control groups. BMI was significantly lower in patients than controls subjects (p=0.001).

Median disease duration was 18 months (range 1 - 312 months) for CD and 31 months (range 1 – 168 months) for UC. CD was ileal in 15.8% of patients, ileocolonic in 68.4% and colonic in 15.8%. For UC, 33.3% of patients had left sided colitis, 42.9% had pancolitis and 23.8% proctitis. Mild and moderate activities of the disease were observed in 73.68% and in 80.9% of the CD and UC patients, respectively. Intestinal resection had been performed in 26.31% of patients with CD and one patient with UC had undergone coloproctectomy with ileoanal anastomosis.

Twenty-two (19 CD and 3 UC) patients had received corticosteroid treatments. The cumulative dose was 3770 ± 1081mg.

Ten patients had received 5-aminosalicylic acid (5-ASA), eight patients azathioprine, one cyclosporine and three infliximab. The results of biochemical markers measured in both groups of patients and controls, and the mean levels are summarized in Table **3**.

Mean calcium, phosphorus and alkaline phosphatase levels were similar in both groups. Median 25-hydroxyvitamin D levels were significantly lower in IBD patients compared with controls (p=0.0001).

Median serum osteocalcin levels were lower and median parathyroid hormone levels were higher in IBD patients compared with the control group but the differences did not reach statistical significance (p = 0.34 and p = 0.187 respectively).

Median urinary Cross Laps levels were significantly higher in IBD patients compared with healthy controls (p = 0.007).

During the follow-up period, none of the patients reported a history or symptoms of bone fracture.

The Median SD of femoral neck and lumbar spine T-scores in IBD patients were -1 (-3.2; - 1.4) and -1.65 (-4; -1.5).

Using the WHO's diagnostic criteria, 12 (30%) IBD patients were classified as normal, 13 (32.5%) were osteopenic and 15 (37.5%) were classified as osteoporotic.

Among controls, 20 subjects (62.5%) had normal BMD, 11 subjects (34.4%) had osteopenia and only one subject had osteoporosis.

Osteoporosis was more frequent in IBD patients than controls (p=0.0001).

No significant difference between UC and CD patients concerning the prevalence of osteopenia or osteoporosis was found (p = 0.828).

Mean age of patients with low BMD (46.42 ± 14.33 years) was significantly higher compared with those with normal BMD (39.75 ± 10.42) (p = 0.001). Fifteen patients had osteoporosis: ten men and five women. The prevalence of osteoporosis was significantly higher in men than women (p = 0.05).

There was no correlation between disease duration and disease clinical activity or disease localization and low BMD. Mean serum level of ESR was significantly higher in IBD patients with low BMD compared with IBD patients with normal BMD (p = 0.039). There was no significant correlation between low BMD and 25-OH vitamin D levels or urinary Cross Laps levels.

Among the twenty-eight patients who had low BMD, 16 had received corticosteroids and 12 did not. In the normal BMD group, 6 had received corticosteroid and 6 did not. The percentage of IBD patients who had received corticosteroid was numerically higher in the low BMD group compared with the normal BMD group but the difference was not statistically significant. The median cumulative dose of corticosteroid in patients with low BMD (515 mg (0 – 11200 mg)) was higher compared with those with normal BMD (250 mg (0 – 23900 mg) but the difference was not statistically significant (p = 0.642).

Table **4** shows the risk factors associated with low BMD in IBD patients.

Table 3. Bone biochemical markers and bone mineral density in the two groups of patients and controls.

Biochemical Markers	IBD Patients (n = 40)	Control (n = 32)	p
Calcium (mmol/l)*	2.43 ± 0.13	2.42 ± 0.10	0.730 ¶
Phosphorus (mmol/l)*	1.22 ± 0.21	1.13 ± 0.20	0.060 ¶
Alcaline phosphatase (UI/l)*	101.25 ± 44.10	88.75 ± 44.18	0.230 ¶
25-OH Vitamin D (ng/ml)**	3.14 (3 – 36.68)	9.03 (3 – 41.74)	0.0001 ¥
PTH (pg/ml) **	47.04 (23.37 – 223.4)	41.57 (22.08 – 83.32)	0.187 ¥
Serum osteocalcin (ng/ml) **	22.36 (8.08 – 60.48)	24.6 (10.66 – 165.6)	0.340 ¥
Urinary CTX (µg/mmol de créatinurie) **	465.06 (28.02 – 876.62)	343.29 (37.77– 1323.53)	0.007 ¥
Osteopenia(%)	13(32.5%)	11(34.4%)	0.45£
Osteoporosis(%)	15(37.5%)	1(3%)	0.001£

* Mean concentration; **Median concentration; ¶ Student t- Test; ¥ U- Mann-Whitney test. £Chi-squared test PTH: Parathyroid hormone; CTX: Cross Laps

Table 4. Risk factors associated with low BMD in the IBD patients.

Risk Factors	Low BMD	Normal BMD	Control Group	p
Gender: Male: N (%) Female: N (%)	13 (32. 5) 15 (37. 5)	4 (10) 8 (20)	15(47) 17(53)	NS §
Age (years)*	46.42 ± 14.33	39.75 ± 10.42	44,72 ± 14,12	NS ¶
BMI (kg/m^2)*	20.71 ± 3.06	21.52 ± 4.59	25.05± 3,96	0.001 ¶
Type of IBD: CD: N (%) UC: N (%)	14 (35) 14 (35)	5 (12.5) 7 (17.5)		NS §
IBD duration (months)*	58.41 ± 86.23	45.92 ± 54.75		NS ¥
Corticosteroids: Yes: N (%) No: N (%)	16 (40) 12 (30)	6 (15) 6 (15)	0	NS §
Cumulative dose of steroids (mg)**	515 (0 – 11200)	250 (0– 23900)	0	NS ¥
Bowel surgery: Yes: N (%) No: N (%)	2 (5) 24 (60)	4 (10) 10 (25)	0	NS §
Disease localization (CD): Colonic: N (%) Ileocolonic + ileal: N (%)	2 (10.5) 12 (63.2)	0 5 (26.3)		NS §
Disease localization (UC): Proctitis: N (%) Left side colitis+pancolitis: N (%)	3 (14.3) 11 (52.4)	2 (9.5) 5 (23.8)		NS §
ESR*	49 ± 15.67	31.8 ± 13.1	10	0.039 ¶
Calcium (mmol/l)*	2.43 ±0.11	2.42 ±0.16	2,42 ± 0,10	NS ¶
Phosphorus (mmol/l)*	1.22 ±0.19	1.23 ±0.25	1,13 ± 0,20	NS ¶
Alcaline phosphatase (UI/L)*	108.12 ±45.26	89.8 ±41	88,75 ± 44,18	NS ¶
25-OH vitamin D (ng/ml)**	3,65	3	9.03	0.009 ¥
PTH (pg/ml) **	42.11	55.4	41.57	NS ¥
Serum osteocalcin (ng/ml) **	28.74	18.51	24.6	NS ¥
Urinairy CTX (µg/ mmol urinary creatinine)**	541.58	358.15	343.3	NS ¥
Fractures (%)	0	0	0	NS §

* Mean value; **Median value; ¶ Student t- Test; ¥ U- Mann-Whitney test; § Chi-squared test. N: number; BMD: Bone Mineral Density; BMI: Body Mass Index; IBD: inflammatory bowel disease; CD: Crohn's Disease; UC: Ulcerative Colitis; ESR: Erythrocyte Sedimentation Rate; PTH: parathyroid hormone; CTX: Cross Laps; NS: not significant

4. DISCUSSION

In this study, we found that 70% of Tunisian patients with IBD have low BMD. The prevalence of osteopenia and osteoporosis was 32.5% and 37.5% respectively. We found a high prevalence of low BMD in our patients, which is considered to be one of the highest prevalence rates among previously-published articles. The prevalence of low BMD has been reported in the range of 22–77% in some earlier studies [26 - 32].

The prevalence of osteoporosis (37.5%) in our cohort of Tunisian IBD patients is comparable with that reported in other studies (12-57%) [14 - 20]. Moreover, the proportion of IBD patients with osteopenia was 32.5% which is comparable with the corresponding value reported from other studies 23-67% [14 - 20]. Osteoporosis is typically a multifactorial disease. The pathogenic mechanisms that might contribute to low BMD in IBD patients include advancing age, low BMI, smoking, alcohol abuse, immobilization or inactive lifestyle, the activation of inflammatory cytokines, hypogonadism, malnutrition, intestinal malabsorption of calcium and vitamin D, the use of corticosteroids, and genetic susceptibility [4, 33].

No significant difference between CD and UC was found. Several studies have failed to demonstrate any difference in the prevalence of bone loss between CD and UC [26, 28, 34, 35]. Other studies reported lower BMD in CD compared with UC [6, 27, 36].

Equally important is that this study the high prevalence of low BMD in male patients as compared with females. In agreement with some studies by Jahnsen *et al.*, Robinson *et al.* and Shirazi *et al.* in the 2 patients groups studied (CD and UC), men had lower BMD than women [1, 5, 37].

There is evidence that body mass affects bone density in IBD. Several studies have shown that BMI is correlated with BMD [3, 30, 32, 38, 39]. Our present study did not found any correlation between BMI and low BMD.

Prior investigators have speculated controversies regarding the features of low BMD and corticosteroid use in IBD patients. In some of these studies, corticosteroid therapy appeared as a risk factor of bone loss [27, 29, 30, 40, 41]. Frei *et al.* [30] demonstrated that bone loss is associated with cumulative steroid dose and duration of treatment. The main mechanism by which corticosteroids induce BMD loss is impairing osteoblast function, inducing osteoblast apoptosis, reducing intestinal calcium absorption and increasing renal

calcium excretion [42, 43].

Unlike other studies, corticosteroid treatment is not associated with low BMD [3, 44, 45]. In the present study, we showed a significant association between duration of treatment and not with cumulative steroid dose.

There is controversial data in the literature regarding bowel resection as a risk factor of low BMD. Von Tirpitiz *et al.* found that history of bowel resection is a significant predictive factor of low BMD [46]. In our present work, in accordance with Silvennoien *et al.* [35] and Stockbrugger *et al.* [47], this correlation could not be found.

Vitamin D deficiency and disturbances of calcium metabolism would be a contributing factor to bone loss in IBD [48]. A review on vitamin D status in IBD reported hypovitaminosis D in up to 65% of adult patients [49]. Several reasons have been suggested for the low vitamin D status in patients with IBD including reduced intestinal absorption of vitamin D as a consequence of ileopathy, disrupted enterohepatic circulation of this vitamin, renal insufficiency and reduced dietary intake and exposure to sunshine [50]. In this study, 25 OHD levels were significantly low in IBD patients compared with controls but were not associated with osteoporosis. Thus, vitamin D deficiency is not the main cause of low BMD in most IBD patients. Moreover, PTH concentrations were higher compared with controls but didn't reach a significant difference. These results are in accordance with those of Robinson [51], Silvennoinen [52] and Haj Taeib [53].

Characterization of bone turnover is useful as previous data showed that biochemical markers of bone metabolism may help to estimate the risk of rapid bone loss, to choose and monitor therapeutic intervention, and to predict long-term osteodensitometric response to therapy [54, 55].

Previous studies using biochemical bone markers in IBD have produced conflicting results, showing either increased bone resorption without a compensatory increase in bone formation including our study [26, 35, 56], decreased bone formation with no variation in resorptive markers [34, 57, 58], decreased [59] or increased [27, 50, 60, 61] both bone formation and resorption markers. We showed an increase in bone resorption with no variation in bone formation as Haj Taeib and al [53] did.

In conclusion, this work can be considered as a warning about the importance of investigating bone changes in IBD patients. Our data set has demonstrated that patients with IBD have a high risk of osteoporosis. Major risk factors for low BMD values were age, inflammation biological marker and duration of corticosteroid treatment. Screening of these patients regularly and supplementation with vitamin D and/or Calcium could prevent osteoporotic process and its complications.

A limitation of our current study might be the restricted population of IBD patients, which needs to be further evaluated in a large cohort of patients. On the other hand, it is interesting to investigate the effect of substitution therapy in these patients by the monitoring of the BMD under treatment.

CONCLUSION

Bone alteration is more frequent in patients with IBD compared with healthy controls whereas there is no difference, concerning BMD, between CD and UC patients. In addition, we showed that serum levels of vitamin D were lower in the IBD group and urinary CrossLaps levels were higher. We also found that Low BMI and hypovitaminoses D in IBD patients due to malabsorption are risk factors for low BMD.

Finally, this study is a warning about the importance of monitoring of BMD by DEXA scan in patients with IBD in the clinical practice. Screening of these patients and starting initiating appropriate treatment could prevent bone loss and its complications especially among at-risk patients.

LIST OF ABBREVIATIONS

IBD	=	Inflammatory Bowel Disease
25(OH) D	=	25-hydroxy vitamin D
PTH	=	Parathyroid Hormone
OC	=	Osteocalcin
CTX I	=	C-terminal telopeptide of type I collagen
BMD	=	Bone Mineral Density
CD	=	Crohn's Disease
UC	=	Ulcerative Colitis
CDAI	=	Crohn's Disease Activity Index
BMI	=	Body Mass Index
ESR	=	Erythrocyte Sedimentation Rate
DXA	=	X-ray Absorptiometry
WHO	=	World Health Organisation
SD	=	Standard Deviations
5-ASA	=	5-Aminosalicylic Acid

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study received research ethics board approval from the "C.P.P.SUD" (0069/2018) committee.

HUMAN AND ANIMAL RIGHTS

No Animals were used in this research. All human research procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013.

CONSENT FOR PUBLICATION

Informed consent was obtained from all participants.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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design, acquisition, and interpretation of data. Khansa CHAABOUNI, Tarek CHAABOUNI and Fatma AYEDI contributed to data analysis.

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