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RESEARCH ARTICLE

STUDY OF POSSIBLE CORRELATION BETWEEN INFLAMMATION AND BONE MINERAL DISORDERS IN CHRONIC KIDNEY DISEASE

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ABSTRACT

Objectives: This study was done to evaluate possible correlation between inflammation detected by the inflammatory marker; high sensitivity C-reactive protein (hs –CRP) and bone mineral disorders in chronic kidney disease patients detected by laboratory and radiological investigations.

Background: Changes in mineral metabolism and bone structure develop early in the course of chronic kidney disease (CKD) and worsen with progressive loss of kidney function. The magnitude of these changes may also be influenced by various therapeutic interventions, such as vitamin D administration and may contribute to such outcomes as fractures, skeletal deformities, and poor growth which persist despite normalization of bone turnover. Chronic inflammatory state in CKD patients due to many underlying factors, including the uremic milieu, elevated levels of circulating proinflammatory cytokines, oxidative stress, carbonyl stress, protein-energy wasting might have possible correlation with bone mineral disorders in chronic kidney disease

Methods: Plasma samples were obtained from CKD patients who were classified into 4 groups 20 patients as control group (stage I,II)and 30 patients (CKD stage III,IV,V)10 patients in each group .The level of hs CRP, alkaline phosphatase, calcium, phosphorus and PTH level were determined in each patient to see if there is possible correlation between high sensitivity C-reactive protein as an inflammatory marker and bone mineral disorders in chronic kidney disease patients

Results: Patients were categorized into groups depending on their estimated GFR by modified diet for renal disease (MDRD) method; there were no significant differences in age, gender and smoking between the four groups. There was positive significant correlation between hs CRP level and alkaline phosphatase, phosphorus level and parathyroid hormone (PTH). However there was no significant correlation with calcium level.

Conclusion: from this study we concluded that there is a possible correlation between hs CPR as an inflammatory biomarker with laboratory and radiological findings in CKD-mineral bone disorders (MBD)

INTRODUCTION

CKD is defined by the presence of kidney damage or decreased kidney function for three or more months, irrespective of the cause. The persistence of the damage or decreased function for at least three months is necessary to distinguish CKD from acute kidney disease. Kidney damage refers to pathologic abnormalities, whether established via renal biopsy or imaging studies, or inferred from markers such as urinary sediment abnormalities or increased rates of urinary albumin excretion. Decreased kidney function refers to a decreased glomerular filtration rate (GFR), which is usually estimated (eGFR) using serum creatinine and one of several available equations.*(1)*

The CKD-MBD is defined as a systemic disorder of mineral and bone metabolism due to CKD that associated with abnormalities of calcium, phosphorus, PTH, or vitamin D metabolism, abnormalities in bone histology, linear growth, or strength, or Vascular or other soft tissue calcification.*(2)*
Traditionally, such lesions have been defined according to alterations in bone turnover, ranging from high bone turnover (secondary hyperparathyroidism, osteitis fibrosa) to lesions of low bone turnover (adynamic bone disease and osteomalacia).*(2)*

The kidney generates the majority of circulating 1, 25(OH) 2D3, converting 25(OH) vitamin D to 1, 25(OH) 2D3 by means of the enzyme 1α-hydroxylase.As renal failure progresses, calcitriol levels and intestinal calcium absorption decline.
However, at the same time, rising PTH levels increase 1α-hydroxylase activity and also release calcium and phosphorus

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from bone, thus maintaining serum calcium levels until late in the course of CKD .[3]

The US National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF-KDOQI) published clinical practice guidelines. The guidelines provided recommended target ranges for various markers of MBD, such as iPTH, total serum calcium and serum phosphate.[4]

Serum alkaline phosphatase levels are commonly elevated in CKD and dialysis patients. The osteoblast is a prominent source of alkaline phosphatase. As hyperparathyroidism and high-turnover bone disease are common in dialysis patients, an elevated serum alkaline phosphatase level is usually considered as a marker of bone disease. However, the potential role of alkaline phosphatase in the pathogenesis of diseases has been increasingly recognized.[5]

Recurrent or chronic Inflammatory processes are common in individuals with chronic renal disease (CKD), including those with chronic renal failure (CRF) and especially end-stage renal disease (ESRD). This is due to many underlying factors, including the uremic milieu, elevated levels of circulating proinflammatory cytokines, oxidative stress, carbonyl stress, protein-energy wasting, enhanced incidence of infections. Although the definition of inflammation is unclear in this setting, CRF-associated chronic inflammation, as assessed by increased C-reactive protein (CRP) levels above 5 mg/L over at least three months.[6]

Deteriorating renal function may enhance overall inflammatory responses because of the decreased renal clearance of factors that are directly or indirectly involved in inflammation. [7]

PATIENTS AND METHODS

The study was inducted at nephrology unit, internal medicine department, Menoufia University Hospital and internal medicine department ,Tanta medical insurance hospital in the period from January 2013 to July 2013 .

The study was conducted on 50 patients classified into 4 groups :

1. **Group 1**: included 10 patients with chronic kidney disease stage 3.
2. **Group2**: included 10 patients with chronic kidney disease stage 4.
3. **Group3**: included 10 patients with chronic kidney disease stage 5.
4. **Group4**: included 20patients (CKD stage I, II) as a control group.

- **Inclusion criteria**

Chronic kidney disease patients (stage I- stage V) before starting renal replacement therapy.

The following patients were excluded from this study

1. Patients have acute infections.
2. Patients have Malignancy.
3. Patients with chronic liver disease.
4. Patients have thyroid gland dysfunctions.
5. Patients have a recent myocardial infarction.
6. Patients have a recent trauma.
7. Patients have a recent physical stress.
8. Patients have non-steroidal anti-inflammatory for last three days, corticosteroids intake, statins intakes.
9. Postmenopausal females.

All studied groups were subjected to the followings

- ***Full history taking***

  Including age, gender, previous medications and duration of diabetes mellitus.

- ***Clinical examination***

  Concering on blood pressure, neurological and cardiac examination.

- ***Radiological***

- **X- ray** on hands to determine bone changes due to bone mineral disease.
- **X- ray** on chest lateral view to determine if there is aortic calcifications

- Echocardiography

Laboratory investigations Include

- Fasting and post prandial blood glucose.
- Alkaline phosphatase level.
- Calcium, phosphorus level.
- iPTH (intact parathyroid hormone)
- SGOT, SGPT.
- Complete blood count.
- ESR.
- Serum Urea.
- Serum Creatinine:(modified rate Jaffemethod).
- Measurement of glomerular filtration rate (GFR) by:

Modification of Diet in Renal Disease (MDRD):
eGFR= 186 X s.Cr –1.154 (mg/dl) X age–0.203(years).
X 1.212 (if African American).
X 0.742 (if female)

- hs CRP
- GGT 9gamma glutamyle transferase)
- Bone biopsy for patient who face criteria for biopsy (low PTH level, low ALP)
- **Principles of hs CRP measurement method**

Serum C-reactive protein (CRP) causes agglutination of the latex particles coated with anti-human C-reactive protein. The agglutination of the latex particles is proportional to the CRP concentration and can be measured by turbidimetry.

Contents and compositions

A. Reagent: 1 x 40 mL.Glycine buffer 0.1 mol/L, sodium azide 0.95 g/L, pH 8.6.
B. Reagent: 1 x 10 mL. Suspension of latex particles coated with anti-human CRP antibodies, sodium azide 0.95 g/L.

DILUTION	1	2	3	4	5
CRP-hs Standard (µL)	30	60	120	180	240
Saline (µL)	210	180	120	60	—
Factor	0.125	0.25	0.5	0.75	1.0

Samples

Serum collected by standard procedures.CRP in serum is stable for 7 days at 2-8ºC.

Calibrations

It is recommended to do a reagent blank every day and a calibration at least every 1 month, after reagent lot change or as required by quality control procedures.

Metrological Charactistics

The following data were obtained using an A25 analyzer. Results are similar with A15. Details on evaluation data are available on request.

Repeatability

Mean concentration	CV	n
2.52 mg/L	1.9 %	20
4.85 mg/L	1.3 %	20

- Reproducibility (run to run):

Mean concentration	CV	n
2.52 mg/L	2.6 %	25
4.85 mg/L	2 %	25

Trueness: Results obtained with this procedure did not show systematic differences when compared with a reference procedure. Details of the comparison experiments are available on request.

Results

This study included 50 predialysis CKD patients classified into 4 groups, allwere subjected to cross sectional study. According to the CKD classification, 20 patients (40%) were in stage 1, 2, 10 patients (20%) in stage 3, and 10 patients (20%) in stage 4, 10 patients (20%) in stage 5. Socio-demographic data (age, gender and smoking) shows no significance **(Table 1).**

Laboratory investigations Showed significant difference regarding blood urea, S.creatnine, FBS and e GFR(MDRD)between all the studied groups while it showed no significant difference regarding SGOT,SGPTand HB level .**Table(3)**

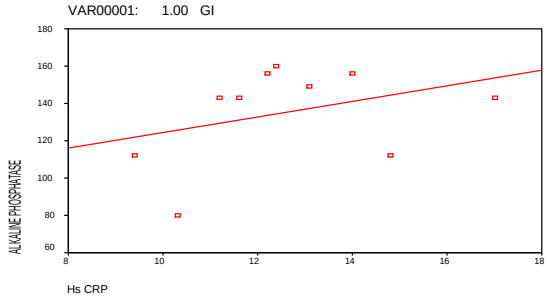


Fig (A -1) Correlation between hsCRP and alkaline phosphatase in group (I)

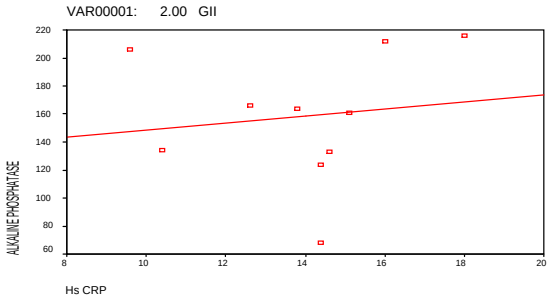


Fig (A -2) Correlation between hsCRP and alkaline phosphatase in group (II)

Concerning bone mineral metabolism, all CKD patients had higher levels of serum CRP (taking 3mg/L as the lower reference limit for long-term inflammation). CRP tended to increase with eGFR decline and there was significant difference between all studied groups regarding iPTH, PO4,alkaline phosphatase level,ESR and hs CRP however total calcium level showed no significant difference between all studied groups. Table(4).

Regarding radiological findings X- ray on hands showed significant difference between all studied groups however X-RAY on chest (lateral view) to show aortic calcification showed no significant difference between studied groups. Table (5)

This study showed that that level of hs CPR and alkaline phosphatase showed positive significant correlation in group (I) and group (II)fig(A-1,A-2),while it showed negative significant correlation in group (III).Table (7) ,Fig (A-3)

Also this study showed that hs CRP and PTH level showed positive significant correlation in all groups.Table (7) ,Fig (B-1,2,3)

Table (1)comparison between all studied patients groups according to age, gender and smoking.

		GI		GII		GIII		Control		X^2	p. value
Age	Range	40-72		44-67		43-72		45-68		**2.435**	**0.096(N.S)**
	Mean +SD	56.3+6.13		55.9+5.74		57.5+6.12		56.4+6.11			
		N	%	N	%	N	%	N	%		
Sex	Male	9	90	7	70	8	80	16	80	**1.250**	**0.741(N.S)**
	Female	1	10	3	30	2	20	4	20		
smoking	smoker	4	40	3	30	1	10	8	40	**1.001**	**0.696(N.S)**
	non smoker	6	60	7	70	9	90	12	60		

Also ,this study showed that 30% of all studied groups is diabetic while 20% of all studied groups are well known to be hypertensive.**(Table2)**

Table (2) Comparison between all studied groups according to medical history .

History	GI		GII		GIII		Control	
	N	%	N	%	N	%	N	%
DM	40	3	30	4	40	4	20	
HTN	10	2	20	3	30	4	20	
DM,HTN	30	1	10	3	30	4	20	
MPGN	10	-	-	-	-	3	15	
polycystic kidney	-	2	20	1	10	1	5	
GN,Crescentic	10	-	-	1	10	-	-	
Obstructive nephropathy	10	-	-	1	10	2	10	
solitary kidney ,DM	-	2	20	-	-	-	-	
Membranous GN	10	1	10	-	-	-	-	
IgA nephropathy	-	-	-	-	-	1	5	
Analgesic nephropathy	10	-	-	-	-	1	5	
Total	100	10	100	10	100	20	100	
X^2	1.336							
P-value	0.529							

Statistics

Statistical presentation and analysis of the present study was conducted, using the mean, standard deviation and chi-square test by SPSS V.16.

1- Mean value $\left(\bar{X}\right)$: the sum of all observations divided by the number of observation:

$$\left(\bar{X}\right) = \frac{\Sigma x}{n}$$

Where Σ = sum & n = number of observations.

2-Standard Deviation [SD]:

It measures the degree of scatter of individual varieties around their mean:

$$SD = \sqrt{\frac{\Sigma \left| x - \bar{x} \right|^{-2}}{n-1}}$$

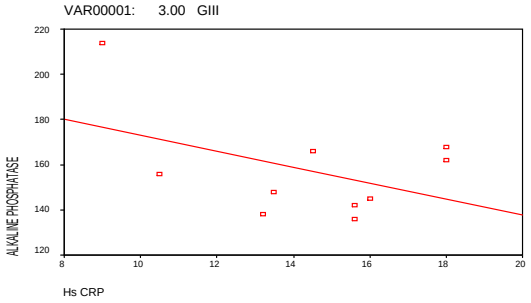
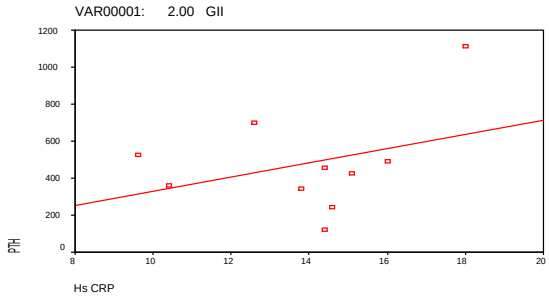


Fig (**B**-1) :Correlation between hsCRP and PTH in group (I)



Fig(B-2) correlation between hsCRP and PTH in group (II)

Fig (**B -3**) :Correlation between hsCRP and PTH in group (III)

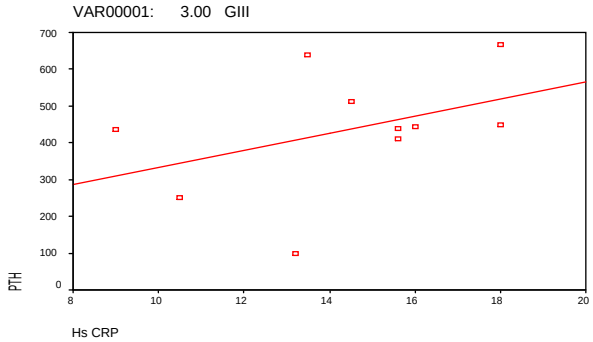


Fig (**B -3**) :Correlation between hsCRP and PTH in group (III)

3. Analysis of variance [ANOVA] tests: According to the computer program SPSS for Windows. ANOVA test was used for comparison among different times in the same group in quantitative data.

4-Chi-square the hypothesis that the row and column variables are independent, without indicating strength or direction of the relationship.Pearson chi-square and likelihood-ratio chi-square. Fisher's exact test and Yates' corrected chi-square are computed for 2x2 tables.

Chi-square test

For comparison between two groups as regards qualitative data.

$$X2 = \Sigma \frac{(O-E)^2}{E}$$

Where:

Σ = Summation.

O = Observed value.

$$E = \text{Expected value} = \frac{\text{vertical total X Horizontal total}}{\text{grand total}}$$

5. Linear Correlation Coefficient [r]:

$$r = \frac{\Sigma \left(X - \bar{X}\right)\left(y - \bar{y}\right)}{\sqrt{\left\{\Sigma \left(X - \bar{x}\right)2\right\}\left\{\Sigma \left(y - \bar{y}\right)2\right\}}}$$

Where :

X= Independent variable.

Y= Dependent variable

DISCUSSION

CKD-MBD refers to the changes in bone, and it is a multifactorial disorder resulting from abnormalities in mineral metabolism, which include vitamin and calcitriol deficiency, hyperparathyroidism, disordered phosphate and calcium metabolism ,and elevated fibroblastic growth factor 23 (FGF23) [(7)].

Historically, the four major types of bone disease that occur in CKD are osteitis fibrosa cystica, low turnover or adynamic bone disease, mixed uremic osteodystrophy(MUO), and osteomalacia. [(8)]

Hyperparathyroidism that develops relatively early in CKD is the major driving force in the development of osteitis fibrosa.[(9)]

The classic findings of hyperparathyroidism in patients with CKD are high turnover with peritrabecular fibrosis, active osteoclasts and increased numbers of multinucleated osteoclasts, woven bone, blurry tetracycline labels, increased cancellous bone volume but decreased cortical thickness, and intratrabecular tunneling.

While earlier reports pointed out a high prevalence of high-turnover bone disease, [(10)] more recent reports have described low-turnover bone disease as the most prevalent disorder in CKD stage 5 patients just before entering dialysis. The bone response to parathyroid hormone (PTH), however, is not consistent, and there is evidence for skeletal resistance to PTH in patients with CKD-MBD.

In this study, there was no statistically significant difference between smoking and level of hs CRP in CKD-MBD , which comes in agreement with *Saito et al., 2004*[(13)].

In the present study, serum SGOT and SGPT levels showed a non-statistically significant difference with BMD, and this is in agreement with *Schiefke et al., 2005*[16], On the other hand *Mokhtar and Hamed, 2006*[17] found that increased serum ALT and AST levels were significantly correlated with decreased BMD in post-menopausal women with post viral cirrhosis (unlike the studied cases in this study).

with SriharshaDamera, Kalani L. Raphael 2011[5] who approved that higher serum levels of alkaline phosphatase were indeed associated with greater prevalence of elevated serum CRP in the CKD and non-CKD populations. The presence of inflammation was related to serum alkaline phosphatase by fitting separate multivariate logistic regression models in the non-CKD andCKD sub-populations.

Table (3) comparsion between all studied patients groups according to laboratory findings.

		GI	GII	GIII	Control	F.test	P-value
HB	**Range**	9.6-13.1	9.3-12.6	8.5-13.1	9.6-12.7	1.609	0.200
	Mean ±SD	11.1±1.06	10.8±1.08	10.3±1.20	11.20±0.69		
Urea	**Range**	40-98	96-212	88-208	42-142	10.36	0.001⋆
	Mean ±SD	69.8±19.3	129.2±33.3	152.6±33.1	106.8±19.7		
Creatinine	**Range**	3.1-6.4	4.8-8.9	3.4-9.6	1.4-7.1	3.996	0.036⋆
	Mean ±SD	4.43±1.05	6.06±1.25	7.78±1.96	2.6.22±1.20		
SGOT	**Range**	3.1-6.4	4.8-8.9	3.4-9.6	3.4-7.1	1.258	0.078
	Mean ±SD	4.43±1.05	6.06±1.25	7.78±1.66	5.22±1.27		
SGPT	**Range**	21-44	38-49	31-49	33-49	2.632	0.096
	Mean ±SD	37.4±6.4	43.1±3.7	39.8±5.4	41±4.6		
FBS	**Range**	77-135	82-312	79-254	100-312	3.625	0.017⋆
	Mean ±SD	97.6±18.2	151.7±79.9	148.6±65.5	201±70.7		
ESTIMATED GFR (by MDRD)	**Range**	28-60	14-29	9-15	63-90	16.352	0.001⋆
	Mean ±SD	49.20+9.63	24.60+5.08	12.30+0.13	72.20+9.33		

Table (4) Comparison between all studied groups according to level of PTH , calcium, phosphorus

		GI	GII	GIII	Control	F.test	P-value
iPTH	Range	61-328	70-1112	100-668	68-436	13.635	0.001⋆
	Mean ±SD	163.8±73.9	377.6±73.3	435±66.5	86.4±70.6		
CA	Range	7.1-9.8	7.7-10.6	7.1-10.2	7.4-11.2	1.632	0.662
	Mean ±SD	9.16±0.87	9.01±1.03	8.60±1.02	8.32±0.91		
Po4	Range	3.6-7.1	4.9-7.4	5.5-8.2	3.6-7.1	2.325	0.024⋆
	Mean ±SD	3.92±0.80	4.14±0.79	6.89±0.88	4.39±0.90		
Alkaline phosphatase	Range	80-160	68-216	136-214	69-124	8.639	0.001⋆
	Mean ±SD	135.4±25.7	155.4±26.1	159.5±30.9	98.4±19.33		
ESR	Range	16-42	18-44	18-43	16-27	5.636	0.002⋆
	Mean ±SD	27.7±8.84	29.4±9.98	30.4±7.30	21±3.61		
Hs CRP	Range	9.4-17	9.6-18	10.9-16	3.8-9	15.632	0.001⋆
	Mean ±SD	12.6±2.23	13.8±2.49	14.3±2.94	6.56±1.26		

Table (5) comparison between all groups according to radiological findings.

		GI		GII		GIII		Control		X^2	p. value
		N	%	N	%	N	%	N	%		
X-RAY on distal forearm and hand.	normal	8	80	5	50	5	50	20	100	6.325	0.026
	subperiostealresorption	2	20	2	20	4	40	0	0		
	Looser zones in those with severe osteomalacia.	0	0	1	10	1	10	0	0		
X-RAY on chest(lateral view) aortic calcification	Yes	0	0	1	10	1	10	0	0	3.52	0.058
	No	10	100	9	90	9	90	20	100		

Table (6) correlation between all studied groups between levels of hs CRP and alkaline phosphatase and PTH.

	Hs CRP					
	GI		GII		GIII	
	r.	p.value	r.	p.value	r.	p.value
ALKALINE PHOSPHATASE	0.363	0.017	0.296	0.028	-0.465	0.185
PTH	0.423	0.011	0.350	0.042	0.414	0.019

C-reactive protein (CRP) is a biochemical by-product produced by hepatocytes, which rises rapidly after an inflammatory stimulus. CRP is the best studied of all the acute-phase proteins, owing to the widespread availability of assays to measure it and well known predictive power of plasma CRP concentrations for future cardiovascular disease (CVD) risk [Carrero JJ, *et al*,2010][18].

Our results showed elevated levels of alkaline phosphatase combined with raised circulating hs CRP level which agreed

osteoblast is a prominent source of alkaline phosphatase. As hyperparathyroidism and high-turnover bone disease are common in dialysis patients, an elevated serum alkaline phosphatase level is usually considered as a marker of bone disease. However, the potential role of alkaline phosphatase in the pathogenesis of diseases has been increasingly recognized. But this disagreed with Wariaghli *et al.,* 2009[11], who showed no significant correlation between alkaline phosphatase level and BMD, This may be due selection of large number of patient is this study.

In this study, Serum calcium showed a non-statistically significant correlation with BMD in the studied group. This result is in agreement with *Karan et al., 2001*[19],*Schiefke et al., 2005*[16] who reported a non-significant correlation between calcium level in BMD and hs CRP as inflammatory markers in their studies. On the other hand *Pongchaiyakul et al., 2008*[20] showed a significant negative correlation between serum calcium when compared with inflammation in osteoporosis.

The finding may be due to the selected subjects included post-menopausal women with a mean age older than that in this study (mean age 45≈44.87) years.

In this study, Serum phosphorus showed a statistically significant correlation with BMD in the studied groups. This result is in agreement with SriharshaDamera, Kalani L. Raphael 2011[(5)]who approved increase level of inflammatory biomarkers with raised phosphorus levels.

Also TetsuMiyamotoa *et al* 2011[(21)] , and Stubbs JR, Idiculla A,2010[(22)] agreed with our results as he approved that Circulating inflammatory biomarkers and polymorphonuclear leucocytes are sensitive predictors of outcome in patients with CKD and are promising primary therapeutic targets and the anti-inflammatory potential of cholecalciferrol supplementation on circulating CRP and TNF **.**

The relationship between serum PTH and inflammation biomarkers such as IL-6 and CRP is not fully understood.

Our results showed significant correlation between iPTH and hs CRP levels which came to agreement with Ahmed AlsayedEmam *et al*, 2011[(23)] who showed that Serum hs-CRP and IL-6 are inflammatory markers associated with an increased risk of cardiovascular disease.

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