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Analysis of Some Demographic, Hematological, and Immunological Parameters among Chronic Hepatitis B Patients with Coronary Artery Disease in Iraq

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ABSTRACT

Background: *Hepatitis B is a major global health problem. It is an infection of the liver caused by the hepatitis B virus. The infection can be acute or chronic.* ***Purpose****: To assess and compare demographic, hematological, and immunological parameters among chronic hepatitis B patients and identify significant clinical patterns and associations.* ***Methods****: A cross-sectional study of 200 hepatitis B patients with coronary artery disease (117males, 83 females) was conducted at Al-Sader Teaching Hospital, Najaf, from November 1, 2023, to December 1, 2024. Serum Blood samples were analyzed for hematological and immunological parameters were measured using ELISA.* ***Results****: Among 200 patients, most were from Al-Najaf (75.5%). Unstable angina was most common (35.5%). Males showed higher hematological values than females, including lymphocytes (p=0.008), RBC (p=0.003), PLT (p=0.006), HB (p<0.001), and WBCs (p=0.002). Diabetic patients had reduced lymphocytes, PLT, HB, and WBCs (all p<0.05) and elevated RBC (p<0.001). Caspase-3 and -9 were significantly higher in asthma, TB, and bronchial allergies compared to those without comorbidities (p<0.001). Smokers had higher caspase-3 (14.2±3.5 vs. 8.9±2.8, p=0.008) and caspase-9 (28.6±4.2 vs. 19.3±3.6, p<0.001) than non-smokers.* ***Conclusion:*** *Chronic hepatitis B in Iraqi patients with coronary artery disease predominantly manifests as unstable angina, with hematological and immunological changes, especially elevated apoptotic markers determined by sex, diabetes, blood group, comorbidities, and smoking. These findings underscore the importance of integrated monitoring and targeted management strategies in this population.*

Keywords: Diabetes, Caspase-3 , Caspase-9 , HBV , Coronary Artery Disease .

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INTRODUCTION

Hepatitis B virus (HBV) is a hepatotropic pathogen that indirectly causes liver disease of variable severity [1]. Due to the interplay between the virus and the host immune system, HBV infection may result in different clinical outcomes, ranging from acute to chronic or fulminant hepatitis, also a significant global health concern, impacting both natural and social sciences [2]. Hepatitis B virus infection continues to be a major global health challenge, with an estimated 254 million people living with chronic hepatitis B as of 2022[3]. Chronic hepatitis is long-term liver inflammation that requires an immediate response to a serious problem. An ongoing problem requires a continuous solution. Inflammation works to defend and repair liver tissues from harm [4,5].

A few studies explored the association between HBV infection and cardiovascular diseases, and conflicting results were reported. Some studies observed that HBV infection was positively associated with coronary artery disease (CAD)[6].Researchers indicate that chronic hepatitis B correlates with an elevated risk of developing atherosclerosis and coronary heart disease, while it is associated with a decreased risk of experiencing an ischemic stroke[7]. Although several studies have evaluated the prevalence and clinical characteristics of chronic HBV among Iraqi patients with CAD [8], few have explored the variations in immunohematological and

apoptotic biomarkers across specific high-risk subgroups such as individuals with diabetes mellitus, different blood groups, smoking status, and family history of metabolic diseases. The present study addresses this gap by assessing and comparing demographic, hematological, and immunological parameters among chronic hepatitis B patients and identifying significant clinical patterns and associations.

METHODS

Study Design: An analytical cross-sectional study involving 200 patients with coronary artery disease who were admitted to Al-Sadr Teaching Hospital and the Al-Najaf Center for Cardiovascular Surgery and Catheterization, Najaf Governorate. Data were collected between November,1, 2023, and December,1, 2024.

All CAD diagnoses were confirmed by cardiologists, and HBV infection was verified serologically. Eligible CAD subtypes included unstable angina, ST-elevation myocardial infarction(STEMI),non-ST-elevation myocardial infarction (NSTEMI), and stable ischemic heart disease. Exclusion criteria were valvular, congenital, or rheumatic heart disease, arrhythmia, and previous COVID-19 infection.

Data were obtained via structured questionnaires that included demographic information such as age, residence, and other information was obtained from the electronic medical records of the heart center.

Sample Size

The sample size was calculated based on the hepatitis B virus prevalence reported in the Kurdistan region of Iraq (3.67%) [9], using the formula [n = Z2P(1–P)/d2]. A confidence level of 95% and a precision of 5% yielded a minimum required sample of 55 participants. To enhance statistical power, account for potential non-response, and improve the precision of estimates, a final sample of 200 participants was recruited. This larger sample ensures more reliable detection of variations in clinical, hematological, and immunological parameters.

Sample Collection

From each patient, 5 mL of venous blood was collected using a syringe, with slow aspiration to minimize hemolysis. Samples were placed in gel tubes and left to coagulate for 10–15 minutes at 25 °C, then centrifuged at 3,000 rpm for 5 minutes. Serum was transferred to Eppendorf tubes and stored at -20 °C until analysis. hematological parameters and immunological, including lymphocytes, red blood cells (RBCs), platelets (PLT), hemoglobin (HB), white blood cells (WBCs), cysteine-aspartic proteases-3 (caspase-3), and caspase-9, were assessed using the ELISA. Diagnosis of chronic HBV by serological markers consists of HBsAg persistence for >6 months, HBeAg presence and/or anti-HBe antibodies, and presence of anti-HBc IgG only.

Ethical Approval

Written informed consent will be obtained from all participants after explaining the study's objectives and before conducting the interview. Ethical approval was granted by the Ethical Committee at the Faculty of Medicine, University of Kufa (Reference #: MEC-16) on 2/14/2024. Additionally, approval was obtained from the Al Najaf Health Directorate.

Statistical Analysis

Data analysis was conducted using SPSS software, version 26. For continuous variables, an independent samples t-test was applied to compare two groups, while one-way ANOVA with LSD post hoc multiple comparisons was used for comparisons among more than two groups. The chi-square test was employed to analyze categorical variables. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

This study included 200 chronic hepatitis B patients. Most of them (75.5%) are from Al Najaf, 25% of them aged less than or equal to 42 years, and more than half of the patients (58.5%) are males, Table (1).

Table (1): Demographic distribution of chronic hepatitis B participants.

Variable	Frequency (No.)	Percentage (%)
Sex		
Male	117	58.5%
Female	83	41.5%
Age (year)		
<= 42	50	25.0%
43 - 47	32	16.0%
48 – 58	42	21.0%
59 - 63	37	18.5%
64+	39	19.5%
Residence		
Al Najaf	151	75.5 %
Aldiwaniyah	22	11%
Al- Basra	8	4%
Karbala	5	2.5%
Baghdad	6	3%
Tikrit	2	1%
Babil	4	2%
Mosul	2	1%
Blood Group		
O+	90	45%
A+	36	18%
B+	30	15%
AB+	31	15.5%
O -	2	1%
A -	2	1%
B -	4	2%
AB-	5	2.5%
Past medical history		
No Past medical history	178	89%
Kidney failure	6	3%
Malignant	2	1%
Asthma	2	1%
Surgery	2	1%
TB	2	1%
Bronchial allergies	2	1%
Psoriasis	2	1%
Hypertension	2	1%
Bowel obstruction	2	1%
Smoking patients		
Smoking	61	30.5%
Non-smoking	139	69.5%

Table 2 showed that acute coronary syndrome (unstable angina) was most prevalent (35.5%, n=71), followed by ST-segment elevation myocardial infarction (STEMI) (28.5%, n=57), non-ST-segment elevation myocardial infarction (NSTEMI) (16%, n=32), and stable chronic ischemic heart disease (20%, n=40).

Table (2): Coronary artery disease patterns in chronic hepatitis B patients.

Type of CAD	N	%
Acute coronary syndrome (unstable angina)	**71**	**35.5 %**
Acute coronary syndrome (myocardial infarction (STEMI))	**57**	**28.5 %**
Acute coronary syndrome (myocardial infarction (NSTEMI))	**32**	**16.0 %**
Stable chronic ischemic heart disease	**40**	**20.0 %**
Total	**200**	**100 %**

Table 3 presents the comparison of five hematological parameters between male and female patients with chronic hepatitis B. Statistically significant sex-based differences were observed for all measured parameters: lymphocytes (males: 3950 ± 430 vs females: 3150 ± 380; $p = 0.008$), red blood cells (RBC; 158 ± 15 vs 132 ± 12; $p = 0.003$), platelets (PLT; 295 ± 40 vs 245 ± 35; $p = 0.006$), hemoglobin (HB; 13.8 ± 1.2 vs 11.5 ± 1.1; $p < 0.001$), and white blood cells (WBC; 10.2 ± 1.5 vs 8.3 ± 1.3; $p = 0.002$). Overall, male patients exhibited consistently higher values across all hematological parameters, with hemoglobin demonstrating the most pronounced sex-related difference.

Table (3): Hematological parameters of patients with chronic hepatitis according to sex of patients.

Blood Test Parameters	Sex		P-value
	Male	Female	
Lymphocyte mean±SD	3950±430** **HS**	3150±380* **S**	0.008** **HS**
RBC mean±SD	158±15** **HS**	132±12* **S**	0.003** **HS**
PLT mean±SD	295±40** **HS**	245±35* **S**	0.006** **HS**
HB mean±SD	13.8±1.2** **HS**	11.5±1.1** **S**	<0.001*** **HS**
WBCs mean±SD	10.2±1.5** **HS**	8.3±1.3* **S**	0.002** **HS**

S: Significant at level 0.05. HS: Highly significant at level 0.01.

The comparisons of hematological parameters between diabetic (n=68) and non-diabetic (n=132) chronic HBV patients, revealing significant differences: lymphocyte counts were lower in diabetics (3200±380 vs 3850±420, p=0.012), RBS levels higher (210±25 vs 98±12, p<0.001), platelet counts reduced (240±35 vs 310±45, p=0.008), hemoglobin levels decreased (11.2±1.3 vs 13.8±1.2, p=0.003), and WBC counts lower (7.5±1.1 vs 9.2±1.3, p=0.018), table (4).

Table (4): The impact of diabetes on hematological parameters in chronic hepatitis B patients.

Blood Test Parameters	Diabetes		p-value
	Yes	No	
Lymphocyte (mean±SD)	3200±380*	3850±420**	**0.012* S**
RBC (mean±SD)	210±25**	98±12**	**<0.001** HS**
PLT (mean±SD)	240±35*	310±45**	**0.008** HS**
HB (mean±SD)	11.2±1.3*	13.8±1.2**	**0.003** HS**
WBCs (mean±SD)	7.5±1.1*	9.2±1.3**	**0.018* S**

S: significant at level 0.05, HS: highly significant at level 0.01.

Hematological parameters across different blood groups in chronic HBV patients are summarized in Table 5, demonstrating significant variations. AB+ patients exhibited the highest lymphocyte counts (4159 ± 2090) and white blood cell (WBC) counts (11.7 ± 4.2), whereas A- patients had the lowest lymphocyte (2224 ± 735) and WBC (6.8 ± 2.7) levels. Platelet counts ranged from 207 ± 68 in A- patients to 333 ± 102 in AB- patients, with A+ (292 ± 99) and B- (324 ± 91) groups also presenting notably elevated values. Hemoglobin levels were relatively stable across groups (12.0 ± 2.2 to 13.6 ± 0.1), although slightly higher levels were observed in A- (13.3 ± 0.5) and O- (13.6 ± 0.1) patients. Random blood sugar (RBS) levels varied between 89 ± 27 (A-) and 171 ± 30 (O-). Statistical analysis confirmed significant differences among blood groups for all parameters, including lymphocytes ($p = 0.008$), RBS ($p = 0.012$), platelets ($p = 0.005$), hemoglobin ($p = 0.021$), and WBCs ($p < 0.001$), with AB+ and B- groups exhibiting particularly distinct hematological profiles.

Table (5): Variations in hematological parameters according to blood group among chronic hepatitis B patients.

Blood group	Blood Test Parameters, Mean±SD				
	Lymphocyte	RBS	PLT	HB	WBCs
O+	3151±787	138±66	254±89	12.0±2.2	8.8±3.7
A+	3 579±502**	157±69	292±99*	12.2±2.4	9.6±4.5
B+	3240±682	137±82	252±87	12.3±2.0	9.1±3.1
AB+	4159±2090***	167±83	288±94	12.0±2.3	11.7±4.2**
O-	3251±334	171±30	282±22	13.6±0.1**	9.7±1.4
A-	2224±735*	89±27*	207±68*	13.3±0.5**	6.8±2.7*
B-	4458±632***	152±52	324±91**	12.3±2.3	13.1±4.4***
AB-	3219±638	123±57	333±102**	10.8±2.9*	9.1±4.9
p-value	**0.008** HS**	**0.012** HS**	**0.005** HS**	**0.021** HS**	**<0.001*** HS**

S: significant at level 0.05, HS: highly significant at level 0.01.

Caspase-3 and caspase-9 levels across different comorbidities in chronic HBV patients with CAD are presented in Table 6, demonstrating significant variation among groups. Patients with asthma exhibited the highest caspase-3 (28.4 ± 3.5) and caspase-9 (45.2 ± 4.8) levels, followed by those with tuberculosis (25.6 ± 2.8 and 42.8 ± 3.9) and bronchial allergies (22.3 ± 3.1 and 47.5 ± 4.2). All comparisons were statistically significant ($p < 0.001$). Notably, asthma, tuberculosis, and bronchial allergy groups had markedly elevated caspase levels ($p < 0.001$), while patients with kidney failure (8.2 ± 5.3 and 12.5 ± 9.8) and malignancies (15.7 ± 10.2 and 18.3 ± 12.6) exhibited moderate but still significant increases.

Table (6): Association between comorbidities and caspase levels in chronic hepatitis B patients.

Past medical history	Caspase levels	
	C.caspase-3 (mean±SD)	C.caspase-9 (mean±SD)
NO chronic dese	10.5±8.1	22.7±17.2
Kidney failure	8.2±5.3*	12.5±9.8**
Malignant	15.7±10.2**	18.3±12.6*
Asthma	28.4±3.5***	45.2±4.8***
Surgery	5.9±4.1**	10.2±7.3**
TB	25.6±2.8***	42.8±3.9***
Bronchial allergies	22.3±3.1***	47.5±4.2***
Psoriasis	6.8±5.2*	15.3±12.4*
Hypertension	18.4±1.5***	28.6±2.3***
Bowel obstruction	9.7±7.8	20.3±15.6
P value	**<0.001*** HS**	**<0.001*** HS**

S: Significant at level 0.05, HS: highly significant at level 0.01.

The differences in caspase levels between smokers (n=61) and non-smokers (n=139) patients are shown in Table 7, revealing significantly higher concentrations in smokers: caspase-3 averaged 14.2±3.5 in smokers versus 8.9±2.8 in non-smokers (p=0.008), while caspase-9 levels were 28.6±4.2 in smokers versus 19.3±3.6 in non-smokers (p<0.001).

Table (7): The impact of smoking status on caspase levels in chronic hepatitis B patients.

Parameters	Smoking		P-value
	Yes N=61	No N=139	
C.caspase-3 (mean±SD)	14.2±3.5**	8.9±2.8**	**0.008** HS**
C.caspase-9 (mean±SD)	28.6±4.2***	19.3±3.6***	**< 0.001*** HS**

S: Significant at level 0.05. HS: highly significant at level 0.01.

Table 8 demonstrates the distribution of IgM3 and IgG3 antibody responses across different subtypes of coronary artery disease (CAD) in patients with chronic hepatitis B. The results indicate a statistically significant association between CAD type and antibody positivity ($p = 0.001$ for both IgM3 and IgG3). Unstable angina patients showed the highest antibody positivity rates, with IgM3 detected in 52.8% and IgG3 in 47.4%.

Table (8): Coronary artery disease types and antibody responses in hepatitis B patients.

CVS	Caspase- 3								
	IgM3					IgG3			
	Positive		Negative		P-value	Positive		Negative		P value
	N	%	N	%		N	%	N	%	
Acute coronary syndrome (unstable angina)	38	52.8	33	25.8		45	47.4	25	23.8	
Acute coronary syndrome (myocardial infarction (STEMI))	22	30.6	35	27.3	**0.001** HS**	24	25.3	32	30.5	**0.001** HS**
Acute coronary syndrome (myocardial infarction (NSTEMI))	8	11.1	24	18.8		16	16.8	25	23.8	
Stable chronic ischemic heart disease.	4	5.6	36	28.1		10	10.5	23	21.9	
Total	72	100	128	100		95	100	105	100	

HS: highly significant at level 0.01.

DISCUSSION

The present study evaluated hematological parameters in patients with chronic hepatitis B according to sex. Analysis of five blood indices revealed statistically significant differences between male and female patients, specifically in lymphocyte count, red blood cells (RBC), platelet count (PLT), hemoglobin (Hb), and white blood cells (WBC). Data consistently show higher values across all parameters in male patients compared to females. The earlier findings show males exhibiting systematically higher values for lymphocyte counts, red blood cell counts, platelet counts, hemoglobin, and white blood cell counts compared to their female counterparts [10,11]. In chronic hepatitis B infection, sex-based differences exist, with males generally experiencing more severe outcomes and a higher likelihood of complications compared to females. These differences are attributed to a combination of immune responses, hormonal factors, and the influence of sex chromosomes [12].These findings highlight significant sex-based variations in hematological profiles among chronic HBV patients, with males exhibiting systematically higher values for lymphocyte counts, red blood cell counts, platelet counts, hemoglobin, and white blood cell counts compared to their female counterparts.

Regarding the Impact of diabetes on hematological parameters in chronic HBV patients. The results showed that significant differences in lymphocyte counts were lower in diabetics, RBS levels were higher, platelet counts reduced, hemoglobin levels decreased, and WBC counts were lower. Statistically significant variations across all measured parameters compared to non-diabetic HBV cases. Hematological changes, such as modifications to red blood cell metabolism, structure, and function (RBCs), the anomalies that T2DM patients confront include (WBCs), platelet counts, and hemostatic parameters [13]. Diabetes was thought to be the primary hazard element for chronic renal diseases, and the synthesis of atherogenic lipoproteins is strongly associated with diabetes and coronary artery disease [14]. The data demonstrates consistent hematological alterations in HBV patients with concurrent diabetes, showing statistically significant variations across all measured

parameters compared to non-diabetic HBV cases. Coronary Artery problems were more common in older diabetic patients than in younger ones [15,16].

Considering variations in hematological parameters related to blood group. Hematological parameters across different blood groups in chronic HBV patients, revealing significant variations: AB+ patients showed the highest lymphocyte count, WBCs, while A- patients had the lowest lymphocytes and WBCs. Platelet counts ranged from A- to AB- with A+ and B-. Statistical analysis confirmed significant differences across blood groups. ABO blood groups have emerged as potential modifiers of disease outcomes, including alterations in hematological indices such as hemoglobin (Hb), white blood cell (WBC) count, platelet (PLT) count, and lymphocyte levels [17]. An earlier study [18] found variations in hematological indices among HBV patients across different blood groups, where blood group B-positive patients showed higher mean Hb and PLT levels compared to other groups. This may reflect differential immune activation or liver pathology progression influenced by blood group antigens [19]. These laboratory results were obtained through standardized blood testing during clinical monitoring, demonstrating clear blood group-related variations in hematological parameters among chronic HBV patients.

According to the association between comorbidities and caspase levels in chronic HBV patients. Caspase-3 and caspase-9 levels across various comorbidities in patients, revealing significant variations. Patients with asthma showed the highest caspase-3 and caspase-9, followed by TB and bronchial allergies. The lowest levels were observed in surgery patients and psoriasis cases. All statistically significant. with asthma, TB, and bronchial allergy groups showing particularly elevated caspase levels, while kidney failure and malignant cases showed moderate. The ILE-CED3 family of enzymes, known as caspase, is divided into two categories: apoptotic (caspase-3/6/7, 2/8/9/10) and inflammatory (caspase-1/4/5/11) [20]. caspase-3-caspase-9 has been confirmed as the central signaling axis in pyroptosis regulation [21]. The relationships between caspase-8 and caspase-9 and pyroptosis in tumor cells have been investigated, revealing a wide variety of possible treatment strategies [22]. Current data show that respiratory conditions (asthma, TB, bronchial allergies) and hypertension were associated with the highest apoptotic activity, while surgical patients and those with psoriasis exhibited the lowest caspase levels among the studied groups [23]. HBV infection comorbidities can influence caspase-3 and caspase-9 levels, potentially affecting the regression in liver disease and total patient results. Specifically, caspase-3, a key executioner caspase in apoptosis [24]. These laboratory measurements were obtained through standardized biochemical assays during clinical evaluations, demonstrating distinct caspase level patterns associated with specific comorbidities in chronic HBV patients. The data show that respiratory conditions (asthma, TB, bronchial allergies) and hypertension were associated with the highest apoptotic activity, while surgical patients and those with psoriasis exhibited the lowest caspase levels among the studied groups.

The analysis demonstrated that chronic HBV patients with a history of smoking exhibited significantly higher concentrations of apoptotic markers, specifically caspase-3 and caspase-9, when compared with their non-smoking counterparts. Specifically, caspase-3 levels were 1.6-fold higher, and caspase-9 levels were 1.5-fold higher in smokers. These statistically significant findings ($p < 0.01$ for caspase-3, $p < 0.001$ for caspase-9) indicate enhanced mitochondrial-mediated apoptosis in smoking

HBV patients, which may exacerbate liver injury and fibrosis progression [25].

Tobacco smoke contains a variety of pro-oxidant compounds that can increase oxidative stress, leading to activation of the intrinsic apoptosis pathway via cytochrome c release, ultimately elevating caspase-9, followed by executioner caspase-3 activation [26]. This mechanism can worsen hepatocellular damage already initiated by chronic HBV infection.

These results align with existing research suggesting that smoking intensifies liver injury and apoptosis in viral hepatitis due to increased oxidative stress and immune dysregulation [25]. The findings show clear smoking-related differences in apoptotic activity markers among chronic HBV patients, with smokers displaying systematically elevated levels of both caspase enzymes compared to non-smokers in this clinical population.

This study assessed the distribution of IgM3 and IgG3 antibody responses in patients with chronic hepatitis B across different categories of coronary artery disease. Patients with unstable angina demonstrated the highest rates of IgM3 and IgG3 positivity, while those with stable ischemic heart disease showed the lowest levels of antibody positivity ($p < 0.001$). These results are consistent with findings from previous studies, which have also reported stronger immune activation in acute coronary syndromes compared to stable ischemic disease [27, 28]. The data demonstrates progressively decreasing antibody positivity from unstable angina to STEMI to NSTEMI to stable ischemic disease, suggesting potential links between inflammatory cardiovascular conditions and humoral immune activation in HBV patients [29]. Previous results were revealing clear associations between cardiovascular disease severity and antibody response patterns in chronic HBV infection [30]. These serological results revealed clear associations between coronary artery disease severity and antibody response patterns in chronic HBV infection.

This study is limited by its cross-sectional design, which prevents causal inference, and by being conducted in a single center, reducing generalizability. Unmeasured confounders, such as therapy status and lifestyle factors, may have influenced results. Finally, the analysis focused only on IgM3 and IgG3, without broader immunological profiling.

CONCLUSION

Chronic hepatitis B infection in Iraqi patients with coronary artery disease most commonly presents as unstable angina. Hematological parameters varied by sex and blood group, while diabetes was linked to reduced lymphocytes, platelets, hemoglobin, and white blood cells. Apoptotic markers were elevated in smokers and patients with respiratory comorbidities, and unstable angina cases showed the strongest antibody responses, reflecting increased immune activation.

Recommendations

It is recommended that chronic HBV patients with coronary artery disease receive regular cardiovascular and hepatic monitoring, with special attention to those with diabetes, respiratory comorbidities, or unstable angina due to their altered hematological and apoptotic profiles. Periodic assessment of immune markers, including antibody responses and caspase levels, may help identify patients at higher risk of severe disease. Management strategies should consider sex- and blood group–related differences and adopt an integrated, multidisciplinary approach to optimize patient outcomes.

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Ethical approval

The present study which is conducted by authors ((Rana Talib Fakher Al-Nafakh and Saif Jabbar Yasir) were approved by the local Department of Ethical approval was granted by the Ethical Committee at the Faculty of Medicine, University of Kufa.

Statement of permission and conflict of interests

The others declare that there is there is no conflict of interest

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