Diagnostic And Differential Efficacy Of Cyclin D1 And Ca15-3 In Breast Cancer And Benign Breast Tumors

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Abstract

Background: Cyclin D1 promotes cancer cell proliferation and is associated with tamoxifen-resistance in breast cancer. The cancer antigen 15-3, CA15-3, was reported to stimulate the body's defense system, but its role in early breast cancer detection remains unclear. Thus, this study sought to investigate the diagnostic and differential utility of cyclin D1 and CA15-3 in breast cancer and benign breast tumors. The study was conducted between April 2022 and January 2023, including 30 breast cancer patients, 30 benign breast tumor patients, and 60 controls. Serum cyclin D1 and CA15-3 levels were measured by ELISA. Receiver operating characteristic curves used to assess the diagnostic performance of each marker. Results: Serum CA15-3 levels were significantly higher in breast cancer patients (38.89±8.63 U/mL) compared to patients with benign breast tumors (32.64±8.47 U/mL) and healthy controls (21.07±8.49 U/mL). In addition, the benign breast tumor group had markedly higher CA15-3 levels than controls. Serum cyclin D1 concentrations differed significantly between the three study groups: 0.85±0.15 ng/mL in breast cancer patients, 0.97±0.21 ng/mL in the benign tumor group, and 0.56±0.14 ng/mL in healthy controls. Conclusion: Elevated cyclin D1 levels were found in breast tumors, suggesting its possible use as a routine diagnostic test. CA15-3 demonstrated the highest levels in breast cancer patients, indicating usefulness for diagnosis and screening.

Keywords: Breast cancer; early detection; diagnostic accuracy; tumor biomarkers

Significance | Investigation of a clinical biomarker in Breast Cancer patients

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1. Introduction

Breast cancer (BC) is the most common malignancy in women worldwide, representing a highly heterogeneous disease with distinct molecular types (Shaath *et al.*, 2021). Molecularly, BC is categorized into three main subtypes based on hormone receptor (ER and PR) and HER2 (ERBB2) status (Dai *et al.*, 2015). Among these, hormone receptor-positive BC accounts for approximately 60%-70% of all cases, while triple-negative BC (TNBC) constitutes about 15%-20%, leaving the remainder as HER2-positive BC subtype (Khan *et al.*, 2021; Vaz-Luis *et al.*, 2013; Yao *et al.*, 2017). Early detection and appropriate treatment are essential in improving outcomes, as BC is the leading cause of cancer-related deaths among women (DeSantis *et al.*, 2019).

Cyclin D1 is a key oncoprotein that regulates cell proliferation and is overexpressed in over 50% of BC cases, predominantly in ER-positive luminal subtypes (Bièche *et al.*, 2002; Casimiro *et al.*, 2012). Despite significant reductions in recuurence of early ER-positive BC with endocrine therapies like such as tamoxifen, approximately one-third of patients develop *de novo* or acquired

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Table 1. Serum levels of cyclin D1 and CA15-3 of participants.

Variables	Control (n= 60)	BBT (n=30)	BC (n=30)	P_1	P_2	P_3
Cyclin D1 (ng/mL)	0.56±0.14	0.97±0.21	0.85±0.15	<0.01*	<0.01*	<0.015*
CA15-3 (U/mL)	21.07±8.49	32.64±8.47	38.89±8.63	<0.01*	<0.01*	<0.034*

P_1= comparison between controls and BBT group, P_2= comparison between controls and BC group, P_3= comparison between BBT and BC group.

Table 2. Serum levels of cyclin D1 and CA15-3 of breast cancer patients based on cancer type

BC type based on hormonal receptor status				
Variables	**Receptor**	**Positive**	**Negative**	**P**
Cyclin D1 (ng/mL)	ER	0.83±0.15	0.91±0.12	0.21
	PR	0.84±0.15	0.87±0.15	0.60
	HER2	0.87±0.07	0.85±0.16	0.77
CA15-3 (U/mL)	ER	38.16±8.79	40.59±8.48	0.49
	PR	38.29±9.38	39.80±7.66	0.64
	HER2	40.70±6.59	38.53±9.05	0.61
BC type based on histopathological classification				
Variables	**Invasive ductal carcinoma (n=18)**	**Invasive Lobular carcinoma (n=3)**	**Ductal carcinoma in situ (n=9)**	**P**
Cyclin D1 (ng/mL)	0.89±0.16	0.81±0.11	0.81±0.12	<0.4
CA15-3 (U/mL)	39.35±8.84	38.44±5.79	38.13±7.71	<0.9

BC= breast cancer, ER= estrogen receptor, PR= progesterone receptor, HER2= human epidermal growth factor receptor 2.

Table 3. Serum levels of cyclin D1 and CA15-3 of breast benign tumor patients based on tumor type

Variables	Fibroadenoma (n=17	Fibrocystic breast changes (n=13)	P
Cyclin D1 (ng/mL)	0.95±0.24	0.98±0.18	<0.75
CA15-3 (U/mL)	34.14±6.48	30.67±10.63	<0.27

Table 4. Diagnostic accuracy analysis of cyclin D1 and CA15-3 in patients and controls. ***(BBT= benign breast tumor; BC= breast cancer; BMI= body mass index)***

BBT vs. BC				
Parameter	**Cut-off value**	**Sensitivity**	**Specificity**	**AUC (%)**
Cyclin D1 (ng/mL)	0.88 ng/mL	43.3%	53.3%	34.1%
CA15-3 (U/mL)	36.07 U/mL	60.0%	70.0%	68.7%
BC vs. control				
Parameter	**Cut-off value**	**Sensitivity**	**Specificity**	**AUC (%)**
Cyclin D1 (ng/mL)	0.70 ng/mL	83.3%	80.0%	91.8%
CA15-3 (U/mL)	28.24 U/mL	90.0%	81.7%	92.8%
BBT vs. control				
Parameter	**Cut-off value**	**Sensitivity**	**Specificity**	**AUC (%)**
Cyclin D1 (ng/mL)	0.72 ng/mL	90.0%	81.7%	95.0%
CA15-3 (U/mL)	26.63 U/mL	80.0%	80.0%	83.5%

resistance (Chang, 2012; Zhao, 2014), which may lead to tumor recurrence and metastasis. Though endocrine treatments target the estrogen signaling pathway in luminal BC, resistance limits their efficacy and new therapeutic targets are needed to improve outcomes (Zhao, 2014). Recent evidence suggests cyclin D1 upregulation may contribute to endocrine therapy resistance and disease progression in luminal breast tumors (Caldon and Musgrove, 2010; Li *et al*., 2020; Musgrove and Sutherland, 2009).

The current gold standard for BC diagnosis is mammography, which has limitations including harmful radiation exposure and low sensitivity for early detection (Smart, 1997; Wang, 2017). Furthermore, needle or surgical biopsies used for diagnosis can be invasive, expensive, time-consuming, and often unnecessary for benign tumors (Bahramy *et al*., 2023). Thus, interest is growing in alternative non-invasive cancer biomarker discovery approaches due to reduced pain, easier sampling, and cost-effectiveness (Bahramy *et al*., 2023; Marrugo-Ramírez *et al*., 2018). Cancer Antigen 15-3 (CA15-3), an FDA-approved BC marker, approved for monitoring metastatic BC and response to treatment (Jäger *et al*., 2000; Sturgeon *et al*., 2008). Increased serum CA15-3 correlates with more advanced BC stages, larger tumors, and positive lymph nodes (Rasmy *et al*., 2016). However, new biomarkers are needed to improve early diagnostic accuracy.

This study aims to assess the diagnostic and differential utility of serum cyclin D1 and CA15-3 levels in distinguishing between women with BC and those with benign breast tumors (BBT). Evaluating these biomarkers may reveal their potential to improve diagnostic accuracy and patient outcomes.

Materials and Methods

A case-control study was conducted at the Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University and Al-Imamain Al-Kadhimain Medical City in Baghdad, Iraq, from April 2022 to January 2023. The study recruited 120 Iraqi women aged 18-65 years, including 30 breast cancer (BC) patients, 30 patients with benign breast tumors (BBT), and 30 healthy controls. The age for BC, BBT, and control groups were 44.76±7.81, 45.53±8.45, and 47.08±7.47 years, respectively. Tumor staging was based on the revised American Joint Committee on Cancer Tumor-Node Metastasis (TNM) classification (Amin *et al*., 2017), and histopathological data were obtained through review of medical laboratory records. Patients with BBT and BC were included based on diagnoses confirmed through clinical examination, mammography, ultrasound, and histological analysis of Tru-cut biopsies. Control participants were selected within the same age range as patients.

The Institutional Review Board (IRB) of the College of Medicine at Al-Nahrain University approved this study. All participants provided written informed consent, and the study was conducted in accordance with the principles of the Helsinki Declaration.

Serum samples were collected at the time of diagnosis, prior to any surgical intervention or therapy, from participants meeting the following criteria: (1) diagnosed with early-stage BC including invasive BC at stages I-III based on clinical examination, imaging, and histopathology; (2) diagnosed with BBT based on clinical evaluation and histology; (3) healthy controls with no personal history of cancer or inflammatory disorders. Patients with any missing information on pathologic stage or histologic grade were excluded.

A 5mL blood sample was drawn at 8:00 AM from each participant, placed in plain tubes and allowed to coagulate at room temperature for 10-20 minutes. The samples then centrifuged at 1500xg for 10 minutes, transferred to new tubes and stored at -70°C until cyclin D1 and CA15-3 assays were performed.

Serum CA15-3 and Cyclin D1 levels were measured using Human CA15-3 (Cat. No. #SL0383Hu, Sunlong Biotech, China) and Cyclin D1 ELISA kits (Cat. No. #SL0559Hu, Sunlong Biotech, China), respectively. Standard curves were established for each biomarker (CA15-3 and cyclin D1) per kit protocols (Supplementary Figures 1 & 2). Serial dilutions were prepared, with 50 µL from each dilution pipetted into corresponding microplate wells. Ten microliters of each sample and 40 µL of sample dilution buffer (1:5 dilution) were added to sample wells without contacting well walls. Wells were gently mixed and incubated for 30 minutes at 37°C with the closure plate membrane in place. The concentrated washing buffer was diluted 30-fold with distilled water. The closure membrane was removed, wells were aspirated and filled with wash solution, held for 30 seconds, then wash solution discarded. This was repeated five times. Fifty microliters of HRP-conjugate reagent was added to each well except the blank control. Wells were sealed with the closure membrane and incubated for 30 minutes at 37°C. The wash process was repeated five times after removing the membrane. Fifty microliters each of Chromogen Solutions A and B were added to each well, gently mixed, and incubated at 37°C for 15 minutes protected from light. To stop the reaction, 50 µL of stop solution was added to each well, changing color from blue to yellow. Optical density (O.D.) at 450 nm was measured using a microtiter plate reader, adjusted to 0 in the blank control well. Readings were completed within 15 minutes of adding stop solution. Finally, CA15-3 and cyclin D1 concentrations in the samples were calculated from the respective standard curves and the original undiluted concentrations were determined by multiplying the calculated concentrations by the dilution factor.

Statistical analysis was performed using SPSS 23 and GraphPad Prism 8. Normality of data distribution was assessed to determine use of parametric (independent t-test, ANOVA) or non-parametr-

Table 5. Spearman correlation analysis of parameters in each group. ***(BBT= benign breast tumor; BC= breast cancer; BMI= body mass index)***

Group		Cyclin D1	CA15-3	Age	BMI
BC	**Cyclin D1**	1	r= 0.656 *p*= <0.001	r= 0.142 *p*= 0.464	r= 0.067 *p*= 0.729
	CA15-3	r= 0.656 *p*= <0.001	1	r= 0.051 *p*= 0.792	r= 0.054 *p*= 0.780
	Age	r= 0.142 *p*= 0.464	r= 0.051 *p*= 0.792	1	r= -0.291 *p*= 0.111
	BMI	r= 0.067 *p*= 0.729	r= 0.054 *p*= 0.780	r= -0.291 *p*= 0.111	1
BBT	**Cyclin D1**	1	r= 0.116 *p*= 0.542	r= -0.077 *p*= 0.684	r= 0.165 *p*= 0.382
	CA15-3	r= 0.116 *p*= 0.542	1	r= -0.294 *p*= 0.114	r= 0.349 *p*= 0.058
	Age	r= -0.077 *p*= 0.684	r= -0.294 *p*= 0.114	1	r= -0.239 *p*= 0.204
	BMI	r= 0.165 *p*= 0.382	r= 0.349 *p*= 0.058	r= -0.239 *p*= 0.204	1
A ls	**Cyclin D1**	1	r= 0.101 *p*= 0.444	r= -0.172 *p*= 0.188	r= 0.090 *p*= 0.493
	CA15-3	r= 0.101 *p*= 0.444	1	r= -0.162 *p*= 0.218	r= 0.143 *p*= 0.275
	Age	r= -0.172 *p*= 0.188	r= -0.162 *p*= 0.218	1	r= -0.152 *p*= 0.254
	BMI	r= 0.090 *p*= 0.493	r= 0.143 *p*= 0.275	r= -0.152 *p*= 0.254	1

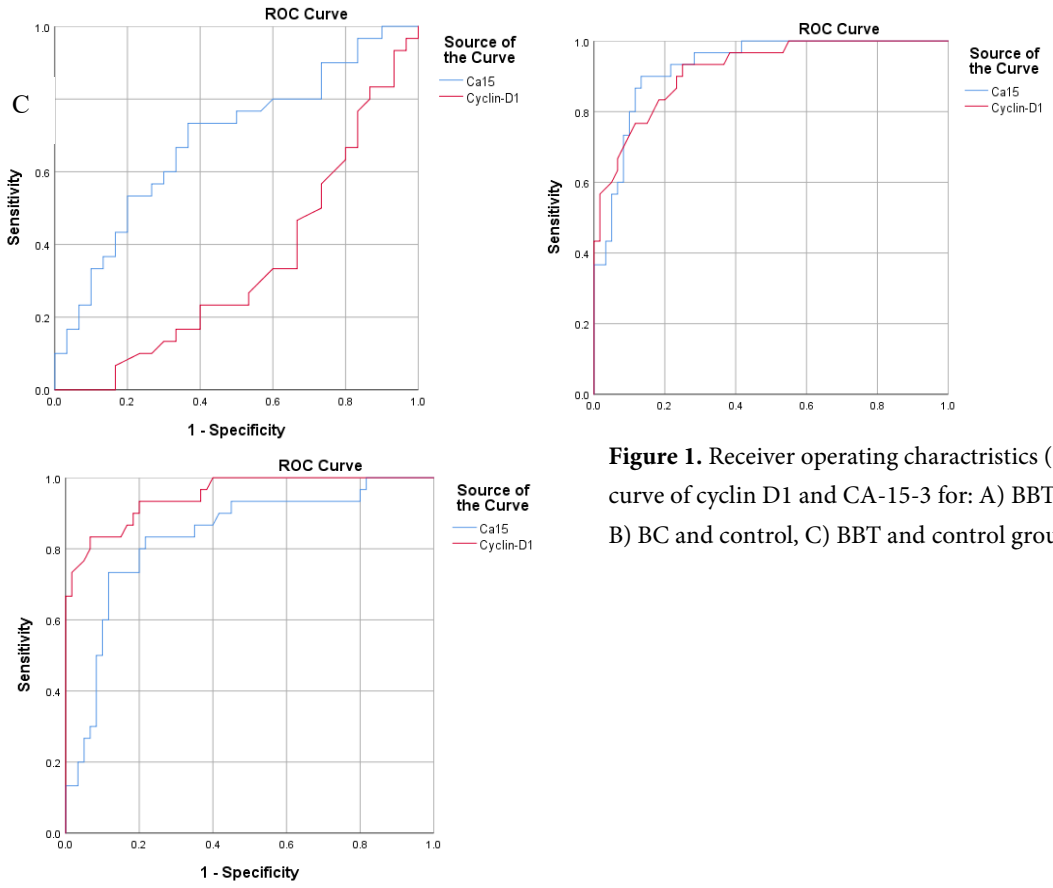

Figure 1. Receiver operating charactristics (ROC) curve of cyclin D1 and CA-15-3 for: A) BBT and BC, B) BC and control, C) BBT and control groups.

(Wilcoxon rank sum) tests for group comparisons. Results were reported as means and standard deviations (SD) for normally distributed data, and as frequencies and percentages (%) for categorical variables. Spearman's coefficient evaluated correlations between factors. A p-value ≤0.05 was considered statistically significant for all tests. Receiver operating characteristic (ROC) curves were generated to assess diagnostic efficiency based on area under the curve (AUC), specificity and sensitivity.

Results and Discussion

Throughout the study period, a total of 120 female subjects were examined and divided into three groups: breast cancer (BC), benign breast tumors (BBT), and control group. The participants were matched for age and BMI (p>0.05). The mean ages for the BC, BBT, and control groups were 44.76±7.81, 45.53±8.45, and 47.08±7.47 years, respectively. The BMIs for the BC, BBT, and control groups were 28.74±1.22, 28.11±1.60, and 27.89±2.06, respectively.

Biochemical analyses were conducted using ELISA to determine the serum cyclin D1 and CA15-3 levels in all patients and control group participants (Table 1). Statistically significant differences were found in cyclin D and CA15-3 levels among the studied groups. The BC group exhibited considerably higher serum CA15-3 levels (38.89±8.63 U/mL) compared to the BBT group (32.64±8.47 U/mL) and the control group (21.07±8.49 U/mL). The BBT group had a significantly higher serum CA15-3 level compared to the control group. Serum cyclin D1 levels were also compared between the study groups, revealing significant differences (p<0.05) between the three groups (Table 1).

The biomarkers were further analyzed in the BC and BBT groups (Tables 2, 3). No significant differences were found in serum cyclin D1 and CA15-3 levels in the BC group based on hormone/HER2 receptor status or histopathological cancer type. Similarly, in the BBT group, no significant differences were observed in serum cyclin D1 and CA15-3 levels depending on the type of tumor.

The diagnostic and differential efficacy of these two biomarkers were estimated using receiver operating characteristic (ROC) curve analysis (Figure 1) as they were significantly higher in BC patients. The cutoff values, sensitivity, and specificity of cyclin D1 and CA15-3 between the BTT and BC groups, BC and control groups, and BBT and control groups were evaluated (Table 4). Both biomarkers demonstrated significant accuracy in detecting BC and BBT.

Furthermore, the Spearman correlation analysis was used to assess the association between cyclin D1 and CA15-3 levels, and age and BMI (Table 5). A significant correlation (p<0.01) was found between the concentrations of CA15-3 and cyclin D1.

Breast cancer (BC) is a prevalent malignancy and leading cause of cancer mortality in women globally. However, advancements in technology and new diagnostic and therapeutic methods have reduced mortality rates (Tarighati *et al*., 2022). The tumor marker CA15-3, an epitope of a large transmembrane glycoprotein MUC-1 and encoded by MUC1 gene, is a protein biomarker that is often elevated in the blood of patients with metastatic BC (Duffy *et al*., 2000; Seale and Tkaczuk, 2022). Serial measurements of CA15-3 levels are used to monitor treatment response and disease progression in advanced BC (Tampellini *et al*., 2006). Increasing CA15-3 levels may indicate treatment failure or recurrence before it is detected on imaging (Seale and Tkaczuk, 2022). In the present study, CA15-3 levels in BC patients were found to be significantly higher compared to BBT patients and controls. Similar findings were observed in studies by Xue *et al.* (2022) and Lian *et al.* (2019), which showed that CA15-3 levels in BBT patients were significantly higher than those in control groups, and serum CA15-3 levels in BC patients were higher than both healthy volunteers and BBT patients (Lian *et al*., 2019; Xue *et al*., 2022). In agreement with these studies, Ławicki *et al.* (2016) also reported statistically significant differences in serum CA15-3 levels between BC patients, healthy females, and BBT patients (Ławicki *et al*., 2016).

The cell cycle regulator cyclin D1 is crucial for G1/S phase transition; its dysregulation can drive abnormal mammary epithelial proliferation (Mohammedi *et al*., 2019). Cyclin D1 is an oncogenic driver in BC and other malignancies (Tchakarska and Sola, 2020). In the current study, serum cyclin D1 levels in the BC, BBT, and control groups were 0.85±0.15 ng/ml, 0.97±0.21 ng/ml, and 0.56±0.14 ng/ml, respectively, with significant differences observed between the groups. Consistent results were reported by Elsheikh *et al.* and Mohammedi *et al*., which reported cyclin D1 overexpression in BC patients relative to those without cancer (Elsheikh *et al*., 2008; Mohammedi *et al*., 2019).

Based on the findings presented and the literature review, there appears to be no significant difference in serum cyclin D1 and CA15-3 levels between different BC subtypes defined by hormone/HER2 receptor status or histopathological classification. In line with our findings, a study by Zhang *et al.* (2019) demonstrated no significant differences in CA15-3 levels among different BC subtypes (Zhang et al., 2019). Moreover, the results shows no statistically significant differences in cyclin D1 levels between ER/PR/HER2 positive and negative BC. Furthermore, the data also showed no significant differences in cyclin D1 or CA15-3 levels between invasive ductal, invasive lobular and ductal carcinoma *in situ* as well as in BBT. This lack of discrimination by histological subtype suggests these markers are not useful for differentiating histopathological types of BC.

The ROC analysis demonstrated the diagnostic accuracy of cyclin D1 and CA15-3 for distinguishing between groups. For BC versus controls, cyclin D1 (AUC 91.8%) and CA15-3 (AUC 92.8%) both showed significant diagnostic accuracy (p<0.001). However, for BBT versus BC or controls, CA15-3 (AUC 68.7% and 83.5%) outperformed cyclin D1 (AUC 34.1% and 95%). For distinguishing between BC and controls, cyclin D1 and CA15-3 demonstrated comparable sensitivity but nearly similar specificity. And, to differentiate BBT from controls, cyclin D1 showed higher sensitivity and nearly similar specificity compared to CA15-3. This indicates cyclin D1 levels are elevated in both benign and malignant conditions compared to healthy individuals. The significant correlation between cyclin D1 and CA15-3 levels further suggests these markers may be similarly upregulated in breast tumors. These results indicate serum cyclin D1 has limited utility for discriminating between benign and malignant breast disease. CA15-3 appears more specific for diagnosing breast cancer versus BBT. Neither marker showed significant correlations with age or BMI.

The small sample size of this study limits the generalizability of the findings, precludes causal determinations, and did not account for potential covariates. To further evaluate the clinical utility of serum cyclin D1 and CA15-3 as diagnostic and prognostic biomarkers in breast cancer patients, large prospective cohort studies that control for relevant confounders are needed in future.

Conclusions

This study provides evidence supporting the diagnostic usefulness of serum cyclin D1 and CA15-3 as biomarkers in breast cancer screening and detection. Significantly higher CA15-3 levels were found in breast cancer patients versus those with benign tumors and healthy controls, with the highest concentrations in the malignant group. Serum cyclin D1 also differed markedly between the breast cancer, benign tumor, and control groups. However, no significant differences were detected in either marker based on breast cancer subtype defined by hormone receptor/HER2 status or histological classification, suggesting limited utility for molecular subtyping or histological differentiation. Though both biomarkers demonstrated accuracy in distinguishing breast cancer from controls, CA15-3 showed superior performance over cyclin D1 for cancer diagnosis and outperformed cyclin D1 for benign versus malignant discrimination. While cyclin D1 exhibited higher sensitivity for separating benign disease from controls, CA15-3 appears more specific for diagnosing breast cancer versus benign tumors. Further large prospective studies are needed to validate the clinical applicability of these serum biomarkers for breast cancer detection and diagnosis.

Author Contributions

Conceptualization: ARR, OFA. Data curation: ARR. Formal analysis: ARR. Funding acquisition: N/A. Investigation: ARR. Methodology: ARR, OFA, OSK, AZA. Project administration: ARR. Resources: ARR, HAA. Software: ARR. Supervision: OFA. Validation: ARR. Visualization: ARR, OFA. Writing – original draft: ARR. Writing – review & editing: ARR, OFA, HAA.

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Competing financial interests

The authors have no conflict of interest.

Ethics

Ethics approval for this study was obtained from the Institutional Review Board Committee at the College of Medicine (IRB-150, dated April 28, 2022). Prior to enrollment, all participants provided written informed consent.

Data availability

Data supporting the findings of this study are available from the corresponding authors (OFA) upon reasonable request.

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