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RESEARCH ARTICLE

Wnt signaling-associated proteins, β-catenin, and E-cadherin as a potential immunohistochemical biomarker of the progression of adenoma to colorectal carcinoma

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ABSTRACT

Background: Colorectal carcinoma (CRC) is the most common and one of the main causes of mortality and morbidity globally among gastrointestinal tract tumors. A benign polyp is the first step in the multistage pathogenesis of colorectal cancer, which eventually progresses to an adenoma and a carcinoma. Wnt/ βeta-catenin signaling pathway plays an initiating and rate-limiting role in colorectal tumorigenesis.

Aim of the work: To evaluate the association between the immunohistochemistry (expression of E-cadherin, and β-catenin with the histopathological grade, and stage of colorectal cancer.

Materials and Methods: The study was retrospectively collected from the archives of the Department of Pathology in Tobruk Medical Center. Eighty-two histopathologically confirmed cases of adenomas (n = 48) (tubular, villous, and tubulovillous), and colorectal adenocarcinoma (Mucinous, and Non-mucinous) (n = 34) were included in this study over two years (2021-2023). The histopathological diagnosis, grade, and staging of the tumors were obtained. While clinical information was obtained from medical records and pathology reports. immunohistochemical staining was performed for all the cases using E-cadherin and β-catenin antibodies, and the results were analyzed.

Results: A total of 82 patients were studied out of these, 51(62.2%) patients were male, whereas 31 (37.8%) were females with a male: female ratio of 1.6:1. Age ranged from 30 years to 80 years. The mean age was the mean age of 52.9 (SD±15.8). A high prevalence of adenoma cases was observed in the age group 30– 40 years. The peak incidence for both types of colorectal carcinoma was in 61-70 years. By scoring the intensity of β-catenin there are significant correlation of β-catenin expression with tumor grade, stage, lymph node metastasis, and types of adenomas. The intensity of staining of E-cadherin in 48 cases of adenomas was showing high expression in 39 cases (81.3%), and low expression in only 9 cases (18.7%). While, the majority of the patients with CRC (58.8%) had low expression of E-cadherin levels, and (41.2%) had high expression.

Conclusion: Our findings imply that E-cadherin and β-catenin may contribute to the invasion and progression of colorectal cancer, which may serve as prognostic indicators for colorectal carcinoma

Keywords: Immunohistochemistry; β-catenin; E-cadherin; Colorectal adenoma; Colorectal adenocarcinoma

Introduction:

Colorectal carcinoma (CRC) is the most common and one of the main causes of mortality and morbidity globally among the many gastrointestinal tract tumors [1]. Generally accepted that CRC is a heterogeneous disease with a wide range of clinical and pathological behaviors, as well as predictions and treatment responses that can vary even among individuals with the same tumor, node, and metastasis (TNM) stage [2]. This might be as a result of the different molecular events linked to the colon tumorigenesis [3]. A benign polyp is the first step in the multistage pathogenesis of colorectal cancer, which eventually progresses to an adenoma and a carcinoma. The slow polyp-cancer progression sequence seen in the general population offers an opportunity to detect and remove the polyps before they undergo malignant transformation [4]. The demand for molecular markers to direct clinical decision-makers on how to categorize patients into the most suitable therapy regiment has never been greater. Earlier in the 1990s, it was determined that mutations in the adenomatous polyposis coli gene (APC) were directly related to the hereditary cancer condition familial adenomatous polyposis (FAP) [5,6]. It rapidly became apparent that the Wnt/ β-catenin signaling pathway plays an initiating and a rate-limiting role in colorectal tumorgenesis as APC mutations were discovered at high frequencies in colorectal adenomas and carcinomas two years after it was first shown that APC and β-catenin interact closely [7]. Wnt/ β-catenin signaling is disrupted in more than 90% of all CRCs, according to recent large-scale exome-sequencing [8].

In brief, when stromal cells and Paneth cells in the intestinal crypt release Wnt proteins, these proteins bind to heterodimeric receptor complexes on the surface of intestinal stem cells (Frizzled/Lrp6) and their effectors, initiating canonical Wnt signaling (i.e., Wnt/ β-catenin signaling) [9]. After that, a signal is sent along a signaling cascade, effectively inhibiting the degradation of cytoplasmic β-catenin [10]. A number of genes involved in proliferation and differentiation are expressed as a result of β-catenin's quick translocation into the nucleus and interaction with DNA-bound TCF/Lef transcription factors [11]. Mutations in APC, β-catenin in cancer cells result in constitutive activation of this signaling pathway, which promotes excessive proliferation and prevents stem cell progenitors from differentiating [12]. Noticeably, β-catenin links E-cadherin to the cytoskeleton in adherens junctions, which is a crucial cellular function [13]. Over the past ten years, numerous datasets at the genetic and protein levels have evaluated the prognostic potential of various Wnt/ β-catenin pathway components in CRC. In particular, deregulation of APC, β-catenin, and E-cadherin has drawn considerable attention [14,15]. In fact, only a few studies have shown that this biomarker has a significant clinical impact. Saldanha et al. indicated that, at low levels of E-cadherin in the cells, E-cadherin sequesters β-catenin at the cell membrane, leading to an increase in MYC and cyclin D1 expression and changing the rate of tumor proliferation [16]. Therefore, this study aims to evaluate the association between the immunohistochemistry (IHC) expression of E-cadherin, and β-catenin. with the histopathological grade, stage of CRC in a subset of patients with primary CR. The results of this research could help in making decision- for newer targeted therapies for CRC.

Materials and Methods

The material of the present study was retrospectively collected from the archives of the Department of Pathology. All surgically resected CRC from 2021 to 2023 were included in the study. Paraffin blocks containing formalin-fixed primary tumors and hematoxylin and eosin-stained slides. The pathology reports of 82 patients of pre-malignant of different types of adenomas (n = 48) (tubular, villous, and tubulovillous), and colorectal adenocarcinoma CRC (n = 34) were collected and the following data such as the histopathological diagnosis, grade, and staging of the tumors were obtained. While clinical information was obtained from medical records and pathology reports. IHC staining was performed for all the cases using E-cadherin and β-catenin antibodies

Processing Procedures:

For each case, a representative paraffin-embedded tissue was chosen. The paraffin wax sections were cut at 4 microns and stained by hematoxylin and eosin stain for routine histopathological examination and immunohistochemical staining by B-catenin and E-cadherin monoclonal antibodies. Each section was obtained from the blocks was placed on positive charge slides, dewaxed in xylene, rehydrated in consecutive descending concentrations of ethanol (100%, 90%, 80%, and 70%), and rinsed in distilled water. For antigen retrieval, slides were placed in a plastic container filled with sufficient citrate buffer pH 6 and heated in a microwave oven at 100ºC for three successive times, five minutes each. The amount of fluid in the container was checked and added if necessary to prevent slides from drying out. The slides were immersed

in 3% hydrogen peroxide for 10 minutes to block endogenous peroxidase.

Immunohistochemical analysis:

Sections were incubated at room temperature with the following Dako Monoclonal Mouse Anti-Human β-Catenin (β-CateninC-1) antibodies (Santa Clara, CA, USA; Catalog number 610154), recognizing a C-terminal epitope between residue 571 and residue 781 of β-catenin. Mouse monoclonal anti-E-cadherin (Clone 36) antibodies were obtained from BD Biosciences (catalog number 610181), recognizing a C-terminal epitope between residue 735 and residue 883 of E-cadherin. Chromogen application by using DAB (3,3-diaminobenzidine tetrahydrochloride). The counterstaining of the sections was done with Mayer's Hematoxylin. Positive and negative controls have been simultaneously run to verify the accuracy of the technique. Slides were scanned by light microscopy and representative fields were selected for analysis. Evaluation: Slides were mounted for light microscope (Olympus BX45 manual microscope, Germany) evaluation of immunoreactivity by a pathologist. The staining of β-catenin and E-cadherin was scored according to the proportion and intensity categories proposed by Allred et al. The intensity score represents their average staining intensity (0 = negative, 1 = weak, 2 = intermediate, and 3 = strong). Staining was evaluated and scored separately for membranous, cytoplasmic, and nuclear staining patterns. Staining score 0 and 1 is considered low expression while staining score 2 and 3 is considered high expression

Statistical Analysis:

The data entry was done using Microsoft Excel (Microsoft Corporation, Redmond, WA). The collected clinical data and histopathological characteristics were analyzed using Microsoft Excel software. Categorical variables were summarized using frequencies and proportions and all results were presented in tabular form and graphs. The groups were tested for statistical significance using the chi-square test and Fisher's exact test, and a p-value less than 0.05 was considered statistically significant

Results:

Clinico-pathological Features:

A total of 82 cases were taken in this study the cases are different types of adenomas (n = 48) (tubular, villous, and tubulo villous), and colorectal adenocarcinoma CRC (n = 34). Age ranging from 30 years to 80 years with a mean age of 52.9 (SD±15.8). The mean age for patients with tubular adenoma (N=18) 47.6 (SD ± 14.6), villous adenoma (N=16) 50.4 (SD ± 15.4), and tubulo-villous adenoma (N=14) 50.8 (SD± 17.9). The maximum number of adenoma cases were observed between the age of 30– 40 years. The mean age for Non-mucinous CRC (N=21) 56.2 (SD ± 16.1), and mucinous CRC (N=13) 60.4 (SD ± 13). The peak incidence was in the 61-70 years for both types of CRC as shown in (Table 1). Most patients were males (62.2%) and (37.8%) were females, with a male-to-female ratio of 1.6:1 (Figure 1).

Table 1: Distribution of adenomas and colorectal carcinoma according to age group.

Age group	***Tubular Adenoma***	***Villous Adenoma***	***Tubulo-villous Adenoma***	***Mucinous Adenocarcinoma***	***Non-mucinous Adenocarcinoma***	
30-40	9	5	6	2	5	
41-50	2	3	1	1	4	
51-60	2	2	2	2	2	
61-70	4	5	3	7	7	
71-80	1	1	2	1	3	
Total No.	**18 (22%)**	**16 (19.5%)**	**14 (17.1%)**	**13 (15.8)**	**21 (25.6%)**	**T=82(100%)**

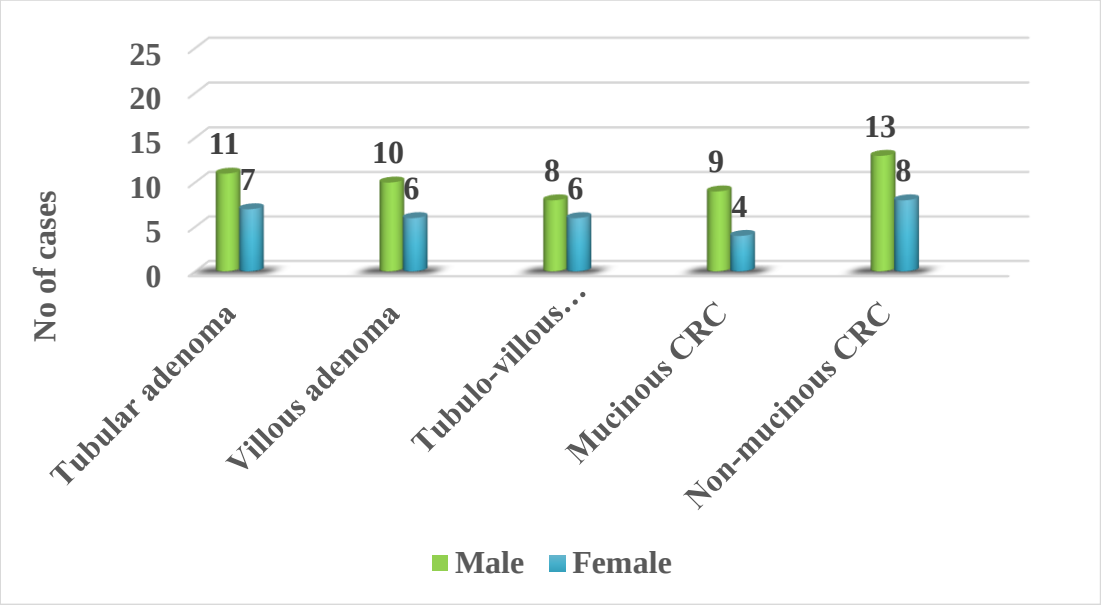


Figure 1: Histopathological pattern of adenoma subtypes and both types of colorectal adenocarcinoma distribution by gender

β-catenin expression

Immunohistochemical examination for determination of β-catenin was accomplished to define subcellular localization and score the all-colorectal neoplasms types included in the study as shows in (Figure 2 first raw). On examination of IHC results and their analysis, the following were distinguished that highest β-catenin membranous expression in benign adenomas; namely tubular adenoma, villous adenoma and tubulo-villous adenoma. There were highest cytoplasmic β--catenin scores noted in low grade colorectal adenocarcinomas and highest β-catenin nuclear score noted in high grade colorectal adenocarcinomas. By scoring the intensity, there was high expression in 27 cases (79.4%) out of 34 malignant adenocarcinoma and low expression in 7 cases (20.6%). While intensity score in 48 cases of adenomas was showing high expression in 12 cases (25%) low expression in 36 cases (75%) as indicated in (Figure 2 second raw).

Univariate analysis revealed significant differences between β-catenin expression (high versus low) in malignant adenocarcinomas and tumor grade, tumor stage and lymph node metastasis (Table 2). And there are significant differences between β-catenin expression (high versus low) and various types of adenomas (Table 3).

Table 2: Correlation between β-catenin and clinicopathologic characteristics of patients with colorectal carcinomas.

Clinicopathologic characteristics	**β-catenin**		***Chi*-square test**
	High expression (27 cases)	**Low expression (7 cases)**	
Grade			
High (9 cases)	5	4	*P*=0.039004*
Low (25 cases)	22	3	
Tumor Stage			
T2 (12 cases)	3	9	*P*=0.012464*
T3 (18 cases)	14	4	
T4 (4 cases)	3	1	
Lymph node metastasis			
Present (13 cases)	8	5	*P*=0.042567*
Absent (21 cases)	19	2	

*p-value <0.05 was considered to be statistically significant.

Table 3: Correlation between β-catenin and various types of adenomas.

Clinicopathologic characteristics	**β-catenin**		***Chi*-square test**
	High expression (12 cases)	**Low expression (36 cases)**	
Adenomas types			
Tubular (18 cases)	3	15	*P*=0.035674*
Villous (16 cases)	2	14	
Tubulo-villous (14 cases)	7	7	

**p*-value <0.05 was considered to be statistically significant.

E-cadherin expression

In contrast to immunohistochemical examination for determination of E-cadherin, that highest membranous and cytoplasmic E-cadherin expression in benign adenomas. There was decrease in E-cadherin expression in high grade colorectal adenocarcinomas. By scoring the intensity, there was high expression in 14 cases (41.2%) out of 34 malignant adenocarcinoma and low expression in 20 cases (58.8%). While intensity score in 48 cases of adenomas was showing high expression in 39 cases (81.3%) low expression in 9 cases (18.7%) as shown in (Figure 2 third raw).

Univariate analysis revealed significant differences between E-cadherin expression (high versus low) in malignant adenocarcinomas and tumor grade, tumor stage and lymph node metastasis (Table 4). And there are significant differences between E-cadherin expression (high versus low) and various types of adenomas (Table 5).

Table 4: Correlation between E-cadherin and clinicopathologic characteristics of patients with colorectal carcinoma.

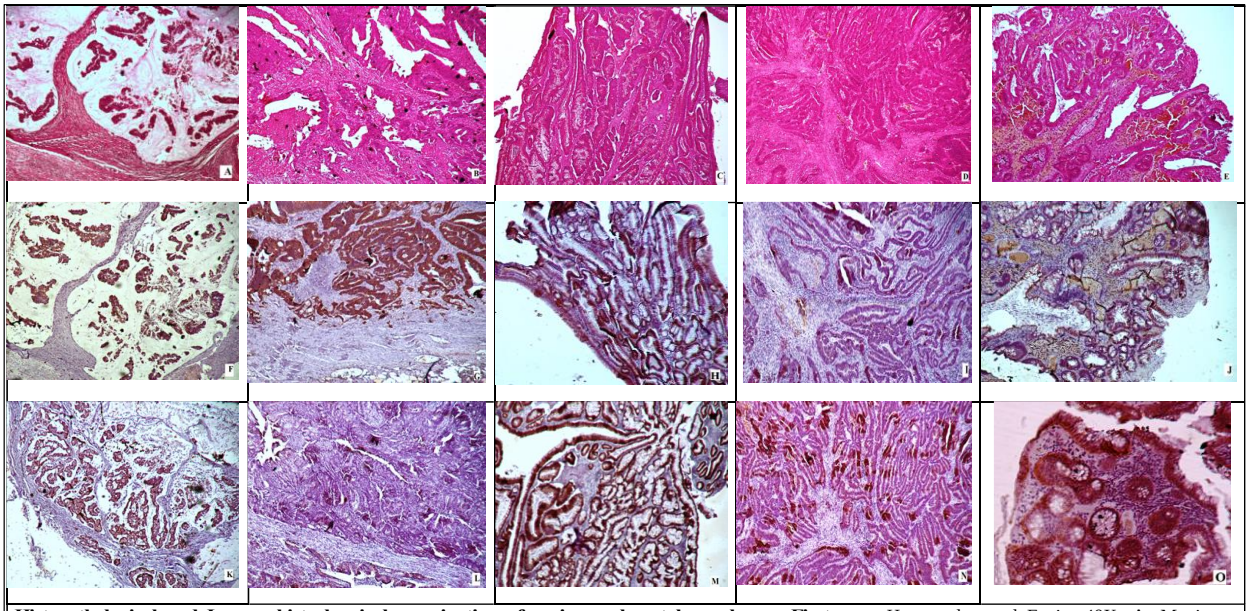
Clinicopathologic characteristics	**E-cadherin**		***Chi*-square test**
	High expression (14 cases)	**Low expression (20 cases)**	
Grade			
High (9 cases)	1	8	*P*=0.032577*
Low (25 cases)	13	12	
T Stage			
T2 (12 cases)	10	2	*P*=0.001059*
T3 (18 cases)	3	15	
T4 (4 cases)	1	3	
LN Mets			
Present (13 cases)	1	12	*P*=0.001801*
Abscent (21 cases)	13	8	

**p*-value <0.05 was considered to be statistically significant.

Table 5: Correlation between E-cadherin and various types of adenomas.

Clinicopathologic characteristics	**E-cadherin**		***Chi*-square test**
	High expression (39 cases)	**Low expression (9 cases)**	
Adenomas types			
Tubular (18 cases)	16	2	*P*=0.021589*
Villous (16 cases)	15	1	
Tubulo-villous (14 cases)	8	6	

**p*-value <0.05 was considered to be statistically significant.



Histopathological and Immunohistochemical examination of various colorectal neoplasms: First row: Hematoxlyn and Eosin, 40X; A: Mucinous Adenocarcinoma, B: Non-Mucinous Adenocarcinoma, C: Tubulo-Villous Adenoma, D: Tubular Adenoma, E: Villous Adenoma. **Second Row:** Beta Catenin Immunohistochemistry, 40X; F: Mucinous Adenocarcinoma, G: Non-Mucinous Adenocarcinoma, H: Tubulo-Villous Adenoma, I: Tubular Adenoma, J: Villous Adenoma. **Third Row:** E-Cadherin Immunohistochemistry, 40X; K: Mucinous Adenocarcinoma, L: Non-Mucinous Adenocarcinoma, M: Tubulo-Villous Adenoma, N: Tubular Adenoma, O: Villous Adenoma.

Discussion:

In this work, using immunohistochemical analysis, we aimed at determining whether Wnt signaling-associated proteins, β-catenin, and E-cadherin were related to progression of colorectal cancer and verify whether it could be used as a prognostic indicator. One of the fundamental goals of translational research in colonic adenoma is to distinguish the small number of individuals who progress to CRC from the majority who do not. Currently, periodic colonoscopic biopsies with histological assessment of adenomas are used to assess the risk of progression to CRC in patients with adenomas [17]**.** A multifunctional protein known as β-catenin is essential for the Wnt/β-catenin signaling pathway and in cellular adherens. Increased expression of nuclear β-catenin has been suggested to be a sign of an aberrant activation of the β-catenin signaling pathway and could play a role in tumor progression [18]. Since colorectal tumorigenesis occurring as a result of mutation in the APC gene (85% cases) follows a gradual multistep sequence, β-catenin expression by immunohistochemistry (nuclear positivity) can be used to determine the malignant potential of colorectal polyps and adenomas [19]. In a recent study, Mårtensson A et al. revealed a correlation between elevated nuclear β-catenin levels and a poor prognosis in CRC [20].

In the present study, the mean age was 52 years with peak incidence was in the 61-70 years for both types of adenocarcinoma, which was similar to findings from other studies [21,22]. In the current study, 62.2% were of the male gender, while 37.8% were female. Consistent with the studies by Iseki et al.[22], Gomaa et al.[23], and Melincovici et al.[24] had a male predominance. Conversely, the studies by Choi et al.[21] and Tunuguntla et al.[25] show female preponderance. In the present study, 52.9% of malignant cases were considered T3 stage, and similar findings were observed by Gomaa et al.[23], Melincovici et al.[24] and Tunuguntla et al.[25], whereas the T4 stage was the major type in the study by Iseki et al.[22].

Histological subgroupings of adenomas are associated with different capabilities to develop malignancy. The three main histological subtypes (tubular, villous, and tubulovillous) of adenomas, were included to provide insights into the expression pattern of β-catenin, and E-cadherin. Our data revealed most of benign adenomas had high expression of E-cadherin
, while most of malignant adenocarcinomas had low E-cadherin expression. There are significant correlation with tumor grade, tumor stage and lymph node metastases. This result is corresponding to Choi et al[21]. and Melincovici et al [24] found a significant association between the loss of

expression of E-cadherin and cancer grading. Tunuguntla et al.[25] shows significant correlation with tumor staging and lymph node metastases. Similarly, in another study, loss of E-cadherin being indispensable in transformation from adenoma to carcinoma sequence in CRC [26]. Lugli et al., demonstrate that nuclear β-catenin expression and loss of E-cadherin membranous expression could be adverse independent prognostic markers in colon carcinoma [27].

Increased expression of β-catenin showed a strong association with increasing grading and staging of the tumor with lymph node metastases, which was also seen in the studies by Choi et al. [21] and Tunuguntla et al. [25] and decreased expression in adenomas. However, no such association was found in other studies by Gomaa et al.[23]

Our result showed the gradual intracellular translocation of β-catenin from a membranous to a nuclear expression in a stepwise manner, following the polyp-adenoma-carcinoma sequence, which is in concordance with the findings of Wong et al.[28] In this study a statistically significant changes in the subcellular localization and increase in β-catenin expression was noted between benign and malignant neoplasms, with the subsequent presence of a higher nuclear β-catenin score in the malignant neoplasms.

In the studies by Bhattacharya et al.[29], Wong et al.[28] and Kovacs et al [30], a significant positive correlation was accomplished between β-catenin subcellular localization and their corresponding membranous, cytoplasmic, and nuclear score. However, the study by Gomaa et al.[23] revealed that loss of β-catenin expression was significantly related with aggressive behavior, high stage, and distant metastases, and aggressive CRC is associated with a decreased expression of β-catenin.

The present study indicates that both β-catenin, and E-cadherin exhibited different expression pattern among adenomas and CRC cases, could provide a useful marker for disease progression, risk stratification, or monitoring of treatment response. It is however now necessary to validate this result using larger panel of pre-clinical and clinical patients in a multicentre setting in order to investigate large cohorts study. Heterogeneity in patient populations and sampling bias are always a challenge in histopathology.

Limitations of the study

Our study has some limitations. First, a small sample size was used to identify the value of β-catenin, and E-cadherin expression in benign and malignant colorectal neoplasms because of the short study period. Second, there is no follow-up of the patients because of the lack of patient registrations.

Future work

To overcome the subjectivity in interpretation of visual scoring, alternative approaches using automated IHC measurements (IHC profiler) which are precise and produce continuous data are being developed. Additionally, automated IHC profilers considerably improves both intra- and inter-observer agreement. Also, the digital methods for IHC quantifications are ideal for large samples size

Conclusion

From the results of the present study, we can conclude that, the type of histological differentiation of colorectal adenomas and adenocarcinoma is directly correlated with the intensity of expression of β-catenin. While E-cadherin expression showed an inversely correlation. β-catenin and E-cadherin could be used as useful prognostic markers for colorectal adenocarcinoma. A long-term follow-up study will be necessary to identify the clinical value of β-catenin and E-cadherin expression in colorectal adenomas and adenocarcinoma.

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