ORIGINAL ARTICLE

Correlation of E-Cadherin Expression with Clinicopathological Parameters and Its Outcomes among Prostate Cancer Patients in Hospital Kuala Lumpur

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ABSTRACT

Introduction: Aberrant expression of E-cadherin has shown to have correlation with advanced disease of prostate cancer. In this study, we evaluated the potential of E-cadherin as a prostate cancer prognostic marker and determined its correlation with patient outcomes. **Method:** 46 prostate cancer specimens in the form of paraffin-embedded tissue blocks were retrieved from the Histopathology Unit, Department of Pathology, Hospital Kuala Lumpur. The expression patterns of E-cadherin were determined by immunohistochemistry staining. The E-cadherin expression was evaluated and scored as positive (3+) and negative or loss of expression (2+ and 1+). The correlations of E-cadherin expression with patient outcomes which included biochemical failure, disease-free, metastasis and local recurrence were determined. Correlations of E-cadherin expression with the currently used traditional clinicopathological parameters were also evaluated. **Results:** There were significant correlations between E-cadherin expression with biochemical failure (p=0.005) and local recurrence (p=0.003). However, there were no significant correlations between E-cadherin expression with disease-free (p=0.864) and tumour metastasis (p=0.430). Comparing the correlation of E-cadherin expression with the traditional clinicopathological parameters, there were significant correlations between E-cadherin expression with pathological staging (p=0.001), Gleason score (p=0.004) and perineural invasion (p=0.001). However, there was no significant correlation between E-cadherin expression with positive tumour margin (p=0.320). **Conclusion:** These results support the potential use of E-cadherin as a prognostic tool for prostate cancer as well as an additional marker along the currently available traditional clinicopathological parameters.

Keywords: E-cadherin, Prostate cancer, Prognostic marker

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INTRODUCTION

Prostate cancer is the third most prevalent tumour among Malaysian men and there were 677 cases reported in the National Cancer Registry in 2011. About 60% of patients were diagnosed during advanced stage of the disease. Higher incidence was reported in patients after the age of 45 years and cases were highest among Chinese followed by Malay and Indian (1). The stage of prostate cancer determines the prognosis and largely contributes to the patient management and treatment options. In order to determine at which stage the patient is currently in, a set of tests and examinations will be done which include digital rectal examination (DRE), blood serum prostate-specific antigen (PSA) level and transrectal ultrasound (TRUS) prostate biopsy.

Gleason system is an established method in determining the grade for prostate cancer. This system is based on the degree of glandular differentiation and growth pattern of the tumour cells which is assessed by histopathological examination. An addition of a new term called grade group was introduced in the grading system following the ISUP (International Society of Urological Pathology) meeting in 2014 in order to stratify the histological patterns in prostate cancer into prognostically relevant grading (2).

The aggressiveness of prostate cancer varies based on the histological type, Gleason score, percentage of tumour volume and TNM staging. Although more than 80% of prostate cancer patients remain clinically silent and less than 3% are lethal, most of these patients were treated (3). Clinicians are currently looking at the preoperative PSA level, pathologic Gleason score and pathological TNM staging as prognostic indicators of prostate cancer to decide on treatment management. However, these prognostic indicators are recognised as

non-absolute determinants of tumour biology especially if used alone since cancers of similar grade and stage in patients of similar age and race, with similar PSA values, often behave differently (4,5).

Patients with clinically indolent prostate tumours have three treatment options which are active surveillance, radiotherapy and surgery to remove the prostate. In this approach, the tumour is regularly monitored and only treated with radiotherapy or surgery if it grows or becomes more aggressive. These treatments are not cheap and can cause major side effects (3,6). However, to date there is no data published on the amount of prostate cancer screening and treatment cost in Malaysia.

E-cadherin is a cell surface glycoprotein in epithelial tissues. It is a readily available immunohistochemical marker and is an established prognostic marker in other cancers including breast and colorectal carcinoma. A few studies have indicated that E-cadherin showed aberrant expression in prostate cancer tissues (3,7). They suggested that loss of E-cadherin expression indicate an aggressive disease which will lead to tumour metastasis as the tumour cells lose its adhesiveness. Several researchers have also concluded that reduced expression of E-cadherin was correlated with loss of differentiation, increased invasiveness and advanced clinical stage of prostate cancer (7,8,9).

MATERIALS AND METHODS

Patient samples

A total of 46 prostate cancer specimens were retrieved from the paraffin-embedded tissue archive in the Department of Pathology, Hospital Kuala Lumpur (HKL). Cases of prostate adenocarcinoma that had undergone prostatectomy with post-operative period of six months or more were included in this study. For each case, the following clinical information was obtained: age, race, diagnosis, type of initial surgical procedure, PSA levels (pre- and post-operative), date of initial diagnosis, staging and local recurrence, Gleason score, tumour margin, perineural invasion and outcomes of tumour. Outcomes of tumour parameters include biochemical failure, disease-free, local recurrence and metastasis at diagnosis (Table 1). Post-operative serum PSA level of more than 0.1 ng/mL (0.1 µg/L) was defined as biochemical failure. Post-operative disease-free was defined as serum PSA level of less than or equal to 0.1ng/mL (0.1 µg/L) for a minimum of 6-month post-operative period. Local recurrence was defined as presence of radiological evidence or positive biopsy for prostate cancer in the operative bed (11).

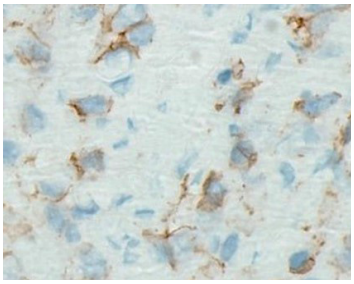
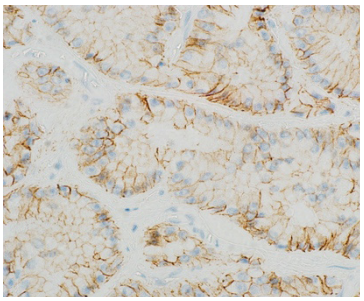
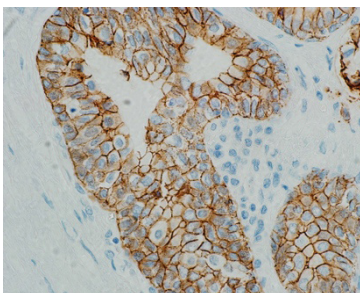
Evaluation

The histological diagnosis of every selected case was confirmed by a histopathologist and the following histopathological features were determined: pathological staging, Gleason score, positive tumour margin and perineural invasion. Tumour grading by Gleason score was classified into two categories as intermediate (score 7 or less) and high (score 8 and above). Pathological staging was also categorised into two categories as low stage/organ confined (pT2) and high stage with extra prostatic extension (pT3).

Immunohistochemistry analysis

Immunohistochemical staining for E-cadherin was performed following pressure cooker antigen retrieval (citrate buffer; pH 6.0), using a mouse monoclonal antibody at 1:150 dilution (Abcam, USA) and the EnVision Plus detection system (DAKO, USA). The slides were then examined under the light microscope and blindly evaluated by two experienced pathologists. The intensity of the colour staining was scored according to the proportion of expression (Table I). E-cadherin expression which showed weak or faint intensity with incomplete membrane staining of >10% tumour cells was scored as 1+ (Fig. 1). Moderate intensity with incomplete circumferential membrane staining of >10% tumour cells or intense with complete and circumferential membrane staining of <10% tumour cells was scored as 2+ (Fig. 2). E-cadherin expression with intense, complete circumferential membrane

Table I: The scoring system for E-cadherin expression

	SCORE	STAINING PATTERN
	1+ (Negative/ loss of expression)	Weak or faint intensity with incomplete membrane staining of >10% tumour cells
	2+ (Negative/ loss of expression)	Moderate intensity with incomplete circumferential membrane staining of >10% tumour cells or intense with complete and circumferential membrane staining of <10% of tumour cells
	3+ (Positive)	Intense, complete circumferential membrane staining of >10% tumour cells

Adapted from (Taghrid Bengallol et al., 2016)

staining of >10% tumour cells was scored as 3+ (Fig. 3). E-cadherin immunoreactivity of 3+ score was categorised as positive, whereas those demonstrating 2+ and 1+ scores were categorised as loss of expression or negative. Skin tissue was used as positive control.

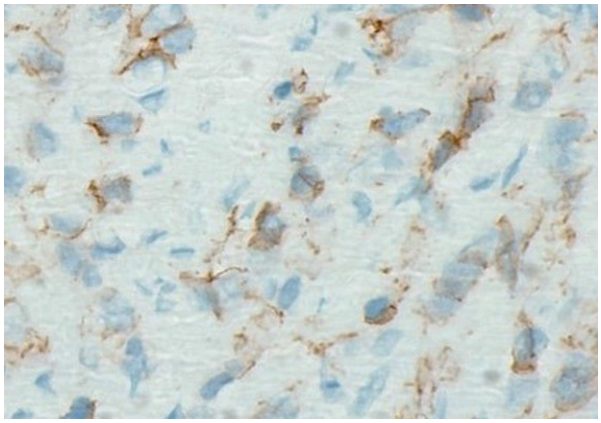


Fig. 1: Score 1+, Weak membranous E-cadherin expression in tumour cells (IHC stain, Magnification x 60). The image only represents the staining intensity but not the percentage.

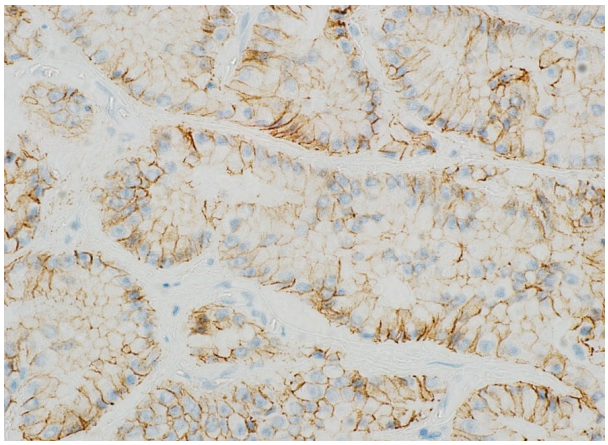


Fig. 2: Score 2+, Moderate membranous E-cadherin expression in cells (IHC stain, Magnification x 40). The image only represents the staining intensity but not the percentage.

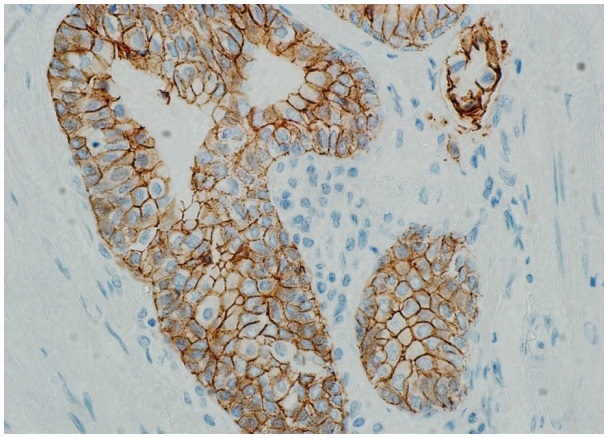


Fig. 3: Score 3+, Strong membranous E-cadherin expression in tumour cells (IHC stain, Magnification x 40). The image only represents the staining intensity but not the percentage.

Statistical Analysis

Statistical analyses were performed using IBM SPSS software version 24. The scores from immunohistochemical staining were categorised as positive (score 3+) or loss of expression (score 1+ and 2+) and determined using Spearman correlation analysis.

Ethics review

Approvals for the study were obtained from the Medical Research Ethics Committee [MREC Ref: NMRR-16-2653-33171(IIR)] and the Ethics Committee for Research of Universiti Putra Malaysia [JKEUPM Ref: FPSK (FR16) P025].

RESULTS

A total of 46 prostate cancer cases were included in this study. The median age of patients was 71 years with age range between 64 to 81 years. Our study showed that the highest frequency of prostate cancer was among Chinese [30 (65%)], followed by Malay [10 (22.0%)] and Indian [6 (13.0%)] (Table II). The race factor was categorised into Chinese and non-Chinese for subsequent statistical analysis. There was no significant correlation between the loss of E-cadherin expression and race groups (p=0.68).

There were 14 (30.0%) cases diagnosed with metastasis at the initial presentation. Post-operatively, biochemical

Table II: Demographic, clinicopathological parameters and outcomes of prostate cancer cases

Parameters	n (%)
Demographic characteristics	
Age	
Range	64 – 81 years old
Median	71 years old
Race	
Chinese	30 (65.0%)
Malay	10 (22.0%)
Indian	6 (13.0%)
Clinicopathological parameters	
Staging	
Low stage/ organ-confined (pT2)	20 (43.5%)
High stage / extra-prostatic extension (pT3a)	26 (56.5%)
Gleason score	
Intermediate (all score 7)	30 (65.2%)
High (all score 8 or 9)	16 (34.8%)
Tumour margin	
Positive	26 (56.5%)
Free	20 (43.5%)
Perineural invasion	
Yes	36 (78.3%)
No	10 (21.7%)
Outcomes	
Biochemical failure	
Yes (PSA > 0.1 ng/mL)	18 (39.0%)
No	28 (61.0%)
Disease free	
Yes (> 6 months post-operative)	30 (65.0%)
No	16 (35.0%)
Local recurrence	
Yes	14 (30.0%)
No	32 (70.0%)
Metastasis (at diagnosis)	
Yes	14 (30.0%)
No	32 (70.0%)

failures with serum PSA level of more than 0.1 ng/mL (0.1 µg/L) during follow-up were seen in 18 (39.0%) cases. Majority of cases (65.0%) were disease-free for a minimum of 6 months duration where the post-operative serum PSA levels were less than or equal to 0.1ng/mL (0.1 µg/L). In this study, local recurrence was identified in 14 (30.0%) cases. Thirty (65.2%) cases had intermediate Gleason scores (all score 7) and 16 (34.8%) had high scores (all score 8 and 9). For pathological staging, 20 (43.5%) cases fell into low stage/organ confined (pT2) while 26 (56.5%) cases were classified as high stage with extra-prostatic extension (pT3a) (Table II).

From 46 cases, 10 (21.7%) cases showed positive expression of E-cadherin with immunohistochemistry score of 3+. The remaining 36 cases showed negative or loss of E-cadherin expression with 15 (32.6%) cases scored as 1+ and 10 (21.7%) cases scored as 2+. There were moderate correlations between loss of E-cadherin expression with biochemical failure (p=0.005) and local recurrence (p=0.003). However, there were no significant associations between the loss of E-cadherin expression and the other outcomes of prostate cancer which were metastasis (p=0.430, rho=0.57) and disease-free (p=0.864, rho=0.57) as in Table III.

Table III: Correlation of loss of E-cadherin expression with outcomes of prostate cancer

Outcome of prostate cancer	E-cadherin	
	rho	p-value
Biochemical failure	0.57	0.005
Disease free	-0.38	0.864
Metastasis	0.17	0.430
Local recurrence	0.57	0.003

The correlations of loss of E-cadherin expression with the traditional clinicopathological parameters (pathological staging, Gleason score, positive tumour margin, perineural invasion) were also analysed. Loss of E-cadherin expression has shown moderate inverse correlations with high pathological stage (rho=-0.71, p=0.001) and high Gleason scores (rho=-0.57, p=0.004) as shown in Table IV. There was also moderate positive correlation (rho=0.69, p=0.001) between the loss of E-cadherin expression and perineural invasion. However, there was no significant correlation (p=0.32) between E-cadherin expression and positive tumour margin.

Table IV: Correlation of loss of E-cadherin expression with traditional clinicopathological parameters

Traditional clinicopathological parameters	E-cadherin	
	rho	p-value
Pathological staging	-0.71	0.001
Gleason score	-0.57	0.004
Positive tumour margin	0.22	0.320
Perineural invasion	0.69	0.001

DISCUSSION

E-cadherin is a cell adhesion molecule that is expressed in many tissues. It has an important role in normal cell development and growth. Apart from that, it has been suggested to inhibit tumour invasion and prevent distance metastasis (15). The association between E-cadherin expression in certain tumours such as breast cancer has been well established. In recent years, loss of E-cadherin membranous expression was also reported in prostate carcinoma. Decrease or loss of E-cadherin expression, detected either by immunohistochemistry or fluorescence in situ hybridization (FISH) has been consistently associated with higher Gleason grade, larger tumour size, advanced pathological stage, extra-prostatic extension and seminal vesicle invasion (8,12,13,14).

In prostatectomy specimens, there are several indicators to look for in order to predict the outcomes for prostate cancer patients. Among the indicators are biochemical failure, disease-free, local recurrence and distance metastasis. Decrease or loss of E-cadherin expression is known to be associated with higher risk of recurrence, advanced disease and poor outcomes which can act as an adjunct marker in predicting the aggressiveness and prognosis of prostate cancer. Downregulation of E-cadherin expression is shown to be associated with loss of tumour differentiation, higher stage and poor prognosis of prostate cancer patients (9).

In this study, the age range for prostate cancer was between 60 to 80 years old. Most of them were Chinese (68%), followed by Malay (18%) and Indian (14%). This result was very much similar with the Malaysian National Cancer Registry which reported that Chinese ethnicity had the highest incidence of prostate cancer with a lifetime risk of 1 in 117 (1). However, no significant correlation was found in E-cadherin expression between the Chinese and non-Chinese groups (p=0.68). This was in contrast to a study by Hsu et al. which had demonstrated significant association between the aberrant E-cadherin expression among Chinese as compared to Caucasians (16). This difference may be due to our smaller sample size.

Both Umbas et al. and Taghrid et al. have concluded that decreased E-cadherin expression was associated with biochemical failure in prostate cancer (8,17). In this study, we observed moderate correlation between loss of E-cadherin expression with biochemical failure (p=0.005, rho=0.57). This was also consistent with another study conducted in Taiwan, which had revealed that decreased in E-cadherin expression was correlated with biochemical relapse (18).

There was also moderate correlation between loss of E-cadherin expression with patients presented with local recurrence (p=0.003, rho=0.57). This was in agreement

with a few other studies including Umbas et al. which had shown significant correlation between the degree of tumour local extension and reduced E-cadherin expression (8).

We have found no significant correlations between the expression of E-cadherin with disease-free (p=0.864) and tumour metastasis (p=0.430). Study by Tradonsky et al. has also shown similar findings in terms of tumour metastasis (3). These findings differ from a study by Umbas et al. which found that E-cadherin expression was moderately correlated with tumour metastasis in 76% of prostate cancer patients (8). Another study by Kuczyk et al. also stated that there was significant correlation between loss of expression of E-cadherin and tumour metastasis (14). Ozekinci et al. however have demonstrated similar findings with ours, showing that E-cadherin was unable to predict the prostate cancer metastatic potential (19). The possibility of the discrepancy between these findings might be due to inter-observer variability in evaluating E-cadherin immunohistochemistry expression. Apart from that, it might be contributed by the differences in the study populations, suggestive of disparity in the biology of prostate cancer.

The correlations of E-cadherin expression with the current traditional clinicopathological parameters (pathological staging, Gleason score, positive tumour margin and perineural invasion) were also observed in this study. We have observed significant moderate correlation (p=0.001, rho=-0.71) between loss of E-cadherin expression with pathological staging. This finding was consistent with a study by Umbas et al. which had demonstrated that 63% of prostate cancer patients with stage III disease or more had decreased E-cadherin expression (8). Univariate analysis by De Marzo et al. has shown that there was significant correlation between reduced level of E-cadherin and advanced clinical stage (20). However, Y.S Hsu et al. concluded that there was no significant correlation between the expression of E-cadherin and pathological staging (16).

We categorised the prostate cancer cases into two categories: Gleason score intermediate (score 7 and below) and high (score 8 and more). In this study, reduced level of E-cadherin expression showed moderate inverse correlation with high Gleason score (p=0.004, rho=-0.57). This result was in agreement with Umbas et al., who proposed that this occur as a consequence of loss of E-cadherin function which is responsible for cell-cell adhesion. The loss of E-cadherin function potentially contributes to increase in tumour cell dissociation and subsequently invasive disease (8,9). However, there was another study by Serdar et al., which has shown no correlation between the expression of E-cadherin and Gleason grade (21).

Perineural invasion is a significant clinicopathological indicator to predict the prognosis of prostate cancer (22). We have observed significant correlation (p=0.001) between the loss of E-cadherin expression and perineural invasion, which was in agreement with a study done in Tunisia (23). However, Taghrid et al. have shown no significant correlation between aberrant E-cadherin expression and perineural invasion (17).

Positive tumour margin is said to have an association with an increased risk of biochemical recurrence after radical prostatectomy (24). Our result has shown no significant correlation (p=0.32) between loss of E-cadherin expression and positive tumour margin. From the literature review, there was no single study which correlates these two variables. Positive tumour margin was categorised as low strength of prognostic parameter as compared to other parameters mentioned (25).

As compared to other current traditional clinicopathological parameters, reduced E-cadherin expression was observed to be more frequently associated with poorly differentiated disease (Gleason grade), advanced staging (pathological staging) and perineural invasion. These findings may be explained by the underlying mechanism of E-cadherin, and loss of its functions which lead to dissociation of the tumour cells to become freely infiltrative. Association with high Gleason score is supported by the fact that loss of E-cadherin expression is related to poor differentiation of tumour cells in prostate cancer (8).

In relation to the outcomes of prostate cancer, reduced E-cadherin expression was observed to be significant in biochemical failure and local recurrence. These two indicators are determining factor for the likelihood of developing local recurrence or distant metastasis (19). Therefore, it further supports the finding that reduced E-cadherin expression correlates with the outcomes of prostate cancer which is consistent with other studies (17,18,20). However, it was noted that no significant correlations were seen between E-cadherin expression and disease-free as well as tumour metastasis.

CONCLUSION

In present study, E-cadherin expression was significantly correlated with the clinicopathological parameters (pathological staging, Gleason score grade, perineural invasion) and the outcomes of prostate cancer patients (biochemical failure and local recurrence). These results propose that E-cadherin expression plays a significant role in the prognosis of prostatic carcinoma in our hospital setting. However, in view of this study as a cross-sectional study design and only confined to our hospital cohort, it must be validated with a larger study setting. As for now, it remains as a research tool rather than a diagnostic tool until more researches are published in the future. It is hoped that the use of this biomarker in clinical setting can help in improving the

screening, prognostication as well as treatment and management of prostatic cancer in the future.

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