ERCC1 and claudin-4 expression in gastric cancer tumorogenesis and development have clinical and pathological relevance.

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Abstract:

Background: Gastric cancer is a significant health and social problem with a high risk of local recurrence. The goal of this study was to look at the immunohistochemistry expression of ERCC1 and claudin-4 in gastric cancer, as well as the relationship with clinicopathologic items and survival in gastric cancer, in order to see how these proteins affect progression and tumorogenesis.

Materials and methods: A total of 155 postoperative specimens were collected, analyzed, and utilized to create tissue microarray blocks from patients diagnosed with stomach cancer. Claudin-4 and ERCC1 immunohistochemical expression were investigated and their relationship with clinicopathological characteristics and patient prognosis was determined.

Results: Claudin-4 and ERCC1 were found in gastric cancer tissues in 54.2 percent and 41.3 percent, respectively. Both proteins were shown to be associated with TNM stage, the number of positive lymph nodes (N), and the depth of invasion (T). Despite the fact that ERCC1 had a strong relationship with histological type and grade, as well as Lauren categorization, claudin-4 did not. High claudin-4 expression was linked to a greater survival rate (58.9%) and an increase in OS (24.5 months) and DFS (18.9 months) in the survival study, but ERCC1 had no correlation with life expectancy (except for DFS on multivariate analysis).

Conclusion: The findings of this retrospective analysis show that the ERCC1 gene polymorphism and Claudin-4 have a significant impact on gastric cancer pharmacokinetics and treatment outcome. Also it may be useful biomarkers to predict the clinical outcomes and can select the cases who receive more aggressive protocols.

Keywords: Gastric cancer; ERCC1; Claudin-4; Survival.

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Introduction:

In 2012, gastric cancer (GC) was the 3rd highest cause of cancer death for both male and female, about 8.8% of all cancer deaths worldwide [1]. In Egypt, GC is the 12th frequent cancer for both sexes, accounting for 1.6 percent of all cancer cases in 2015, with an estimated 1271 new patients [2]. The rate of occurrence varies by location in Egypt, with Upper Egypt having the greatest rate (2.48 percent) and Lower Egypt having the lowest rate (0.98 percent).also in the clinical oncology department of Mansoura University, reported 47 cases (1.79%) out of 2620 cases totally at 2015 [3].

Gastric cancer is categorized as either cardia GC or non-cardia GC depending on where it occurs in the stomach (proximal or distal). This geographical categorization is also linked to various risk variables in different parts of the country. The highest rates of GC, whether cardiac or non-cardiac, are seen in Eastern and Southeast Asia [4].

Because of its substantial health and social burden, several researches have attempted to study the major cancer-related survival and death prognostic variables of GC. The local recurrence of stomach cancer, which occurs in half of patients after surgery, is a severe concern. Despite the fact that advanced surgical techniques and adjuvant treatments have improved patient outcomes for GC, most cases are presented at an advanced stage, limiting treatment options [5]. Furthermore, using of molecular subtypes to predict and provide data for treating GC cases may not impress anyone. The identification and definition of new pathways and specific molecular markers that may be

used for diagnosis and therapy is still a tough but necessary task in improving GC outcomes.

Claudins are extracellular transmembrane tight junction proteins that might be used for diagnosing and treatment [6]. Claudin expression variations may result in a dysfunctional tight junction and promote tumorogenesis in certain epithelial malignancies [7]. In addition to inhibiting cell-to-cell adhesiveness, which allows cancer cells to migrate and spread, it also promotes tumor invasion and metastasis [8]. Claudin-4 is a member of the claudin family, and it was originally identified by Gress et al [9] in pancreatic cancer, where it was shown to be overexpressed. Overexpression of Claudin-4 was later discovered in a variety of cancers [10].

Excision repair cross-complementation group 1 (ERCC1), a rate-limiting enzyme in the nucleotide excision repair pathway, is responsible to protect DNA from mutations and other damage. It works through the nucleotide excision and repair pathway, leading to the hypothesis that the amount of expression is a good indication of nucleotide excision repair capacity [11]. Because of its function in the repair of cisplatin CDDP-DNA adducts, ERCC1 is considered to be linked to platinum-based treatment resistance [12]. Thus, overexpression of the ERCC1 gene reduces the efficacy of CDDP-based treatment for many kinds of epithelial malignancies, such as advanced gastric cancer [13]. Most researchers are interested in ERCC1 expression in lung cancer, although there are few studies looking at its function in pancreatic, gastric, and bladder cancer.

ERCC1 is being investigated as a possible biomarker for a variety of malignancies [14]. ERCC1 is being investigated as a possible biomarker for a variety of malignancies [14]. There are limited studies on the ERCC-1 gene's additional activities in tumor cells, including its associations with tumor progression indicators and tight junction proteins in gastric cancer cells.

In this work, we looked at the immune-histochemistry expression of claudin-4 and ERCC1 in primary GC, as well as their relationship with clinicopathologic parameters and survival in GC, to see how these proteins affect tumorogenesis and progression.

Patients and Methods:

Patients:

From January 2008 to January 2018, 155 samples of postoperative tissues from stomach cancer cases were collected from (the Gastroenterology and Oncology Center Mansoura University) in Mansoura, Egypt. Except in selected high-risk instances, all patients got conventional stomach resection and D2 lymphadenectomy based on the original tumor's location [15]. The study excluded patients with insufficient pathologic data and specimens, as well as those who died as a result of the operation. Follow-up data was collected after the operation. Overall survival (OS) and disease free survival (DFS) were calculated.

Histopathology

The research group's formalin-fixed paraffin-embedded tissues blocks were collected from the pathology laboratory archives. The three regions indicative of tumor for tissue microarray (TMA) blocks were marked on the hematoxylin and eosin stained slides. The researchers looked for vascular invasion and perineural invasion. The tumors were categorized using the World Health Organization's 2010 classification system [16], and staged in accordance with (American Joint Committee on Cancer eighth edition of the TNM staging system) [17].

Immunohistochemistry

TMA blocks were produced, and 4-mm-thick sections were stained with monoclonal antihuman claudin-4 (clone 3E2C1, Thermo, dilution 1: 100) and mouse monoclonal anti-ERCC1 against the amino-terminal 304 amino acids (clone CL1249, a dilution of 1: 150, Thermo). Where membrane and cytoplasm staining was confirmed positive, immunohistochemistry (IHC) expression of claudin-4 was assessed. Claudin-4 was divided into 2 groups: low expression, when the proportion of positively stained cells was less than 50%, and high expression, when the percentage of positively stained cells was more than 50%. The negative control for claudin-4 was normal stomach mucosa, while the positive control was colonic mucosa [18].

Nuclear staining was used to assess ERCC1 expression, and the incidence of stained nuclei was categorized as negative (less than 10% of tumor cells) or positive (more than 10% of tumor cells) [12]. In the case of ERCC1, the positive control was human tonsil tissue, whereas the negative control was performed without the use of the main antibody. Without knowing the specific diagnosis of each patient, 2 independent pathologists (K.A. and D.I.) independently examined all of the slides. Cases where the pathologists disagreed were re-evaluated by both of them until a conclusion was made for inconclusive cases.

Statistical analysis

SPSS version 22 has been used to gather and process patient data (Inc, Chicago, IL). Using percent and number, qualitative data were described. Average and range of non-parametric information and mean ± standard deviation for parametric data were described as quantitative data. The Chi-square and Monte Carlo tests were used to compare categorical variables. Survival curves were created using Kaplan-Meier and the log rank test was compared. The multivariate analysis was conducted using the Cox model of proportional hazards. Statistically significant was a value of $p < 0.05$.

Results:

Clinicopathological data

Table 1 summarizes the clinicopathological findings of our 155 patients. The average age was 52.23 years (range, 17-76). 99 cases were men, with a men-to-women ratio of 1.8:1. The following categories were used to categorize the cases: There were 101 (62.5%)

instances of adenocarcinoma "papillary and tubular", 11 (7.1%) patients of signet ring carcinoma, 24 (15.5%) patients of mucinous carcinoma, and 19 (12.3%) patients of undifferentiated carcinoma (Figure 1). Perineural invasion was found in 18.1 percent of the cases, with 94 instances (60.6 percent) in stage II (A & B).

Table1: Patients and pathological characteristics of the study groups

Basic characteristics	Values (%)
Age (year)	
<50	73(47.1%)
≥50	82(52.9%)
Gender	
Male	99(63.9%)
Female	56(36.1%)
Tumor size	
<2	28 (18.1%)
2-5	92 (59.4%)
>5	35 (22.6%)
Site	
Fundus	48 (31%)
Body & antrum	97 (62.5)
Pylorus	10 (6.5%)
Shape	
Fungating	64 (41.3%)
Infiltrating	48 (31%)
Ulcerating	43 (27.7%)
Histologic type	
Adenocarcinoma (Papillary& tubular)	101(65.2%)
Mucinous carcinoma	24 (15.5%)
Signet ring carcinoma	11 (7.1%)
Undifferentiated carcinoma	19 (12.3%)
Grade	
Differentiated (well & moderate)	72(46.5%)
Less-differentiated (poor & undifferentiated)	83(53.5%)
Lauren classification	
Intestinal	104 (67.1%)
Diffuse	51 (32.9%)
Lymphatic invasion	
Yes	55(35.5%)
No	100(64.5%)
Perineural invasion	
Yes	28(18.1%)
No	127(81.9%)
T stage	
T1	7(4.5%)
T2	83(53.5%)
T3	57(36.8%)
T4a	8(5.2%)
N stage	
N0	45(29%)
N1	60(38.7%)
N2	40(25.8%)
N3a	10(6.5%)
TNM stage	
I (A, B)	31(20%)
II (A, B)	94(60.6%)
III (A, B)	30(19.4%)
ERCC	
Negative	64(41.3%)
Positive	91(58.7%)
Claudin-4	
Low expression	71(45.8%)
High expression	84(54.2%)
Follow up data for 119 cases	
Estimated mean Disease free survival	15.9 months
Estimated mean Overall survival	20.9 months
Recurrence	
Absent	91 (76.5%)
Present	28 (23.5%)
Mortality during follow up	62 (52.1%)

Figure 1 shows representative micrograph from the research group, including [(A) poorly differentiated adenocarcinoma, (B) signet ring carcinoma, and (C) mucinous adenocarcinoma (H&E, x200)]. [Claudin-4 immunohistochemistry, x400, shows low expression in poorly differentiated adenocarcinoma (A1) and strong expression in signet (B1) and mucinous carcinoma (C1)]. [Positive nuclear reaction in poorly differentiated adenocarcinoma (A2), negative reaction in signet (B2) and mucinous carcinoma (C2)], immunohistochemical expression of ERCC1, x400.

Immunohistochemistry

Table 2 shows the immunohistochemistry expression of claudin-4 and ERCC1, as well as their relationship to clinicopathological characteristics. Figure 1 shows sample examples of both proteins' expression in several of the research group's histological variants. The depth of less invasion (T2, 40.8 percent), decreased number of positive LN (N0, 52.4 percent), the absence of perineural invasion, and overall lower TNM stage (all P = 0.00) were all associated with high levels of claudin-4 expression. Regardless of the fact that 55.4 percent (56/101) of adenocarcinoma had high claudin-4 expression and 62.5 percent were more differentiated, it had no obvious association with the histology type or grade of the tumor.

Lauren classification (P = 0.00), histological grade and type (P = 0.00), number of positive lymph nodes (P = 0.001), depth of invasion (P = 0.039), total TNM stage (P = 0.001) and different chemotherapy protocols were received (P = 0.001) all exhibited statistical significance for ERCC1 expression. Adenocarcinoma and better differentiated grade were found in 84.6 percent and 65.9% of ERCC1 positive patients, respectively. Both proteins' IHC expressions exhibited a significant correlation, with 71.4 percent of individuals with high claudin-4 expression also having positive ERCC1 expression.

Immunohistochemical expression of claudin-4 and ERCC1 in relation to survival

Only 119 patients (out of 155 totals) have postoperative follow-up data. The average FU time was 20.9 Ms, with a mortality rate of 52.1 percent (62/119, Table 1). We discovered that the group expressing high levels of claudin-4 had a considerably higher survival rate (58.9%, P = 0.023), as well as an increase in OS (24.5 months, P = 0.023) and DFS (18.9 months, P = 0.003), indicating that it can be utilized as a marker of excellent prognosis (Figure 2). Furthermore, positive (ERCC1) expression had no statistical relevance with DFS, OS, or survival status (Table 2).

Fig 2: The Kaplan-Meier curve was used to calculate survival curves for gastric cancer patients based on the expression status of claudin-4 (A, C) and ERCC1 (B, D). There are substantial variations between claudin-4 and ERCC1 expression patterns.

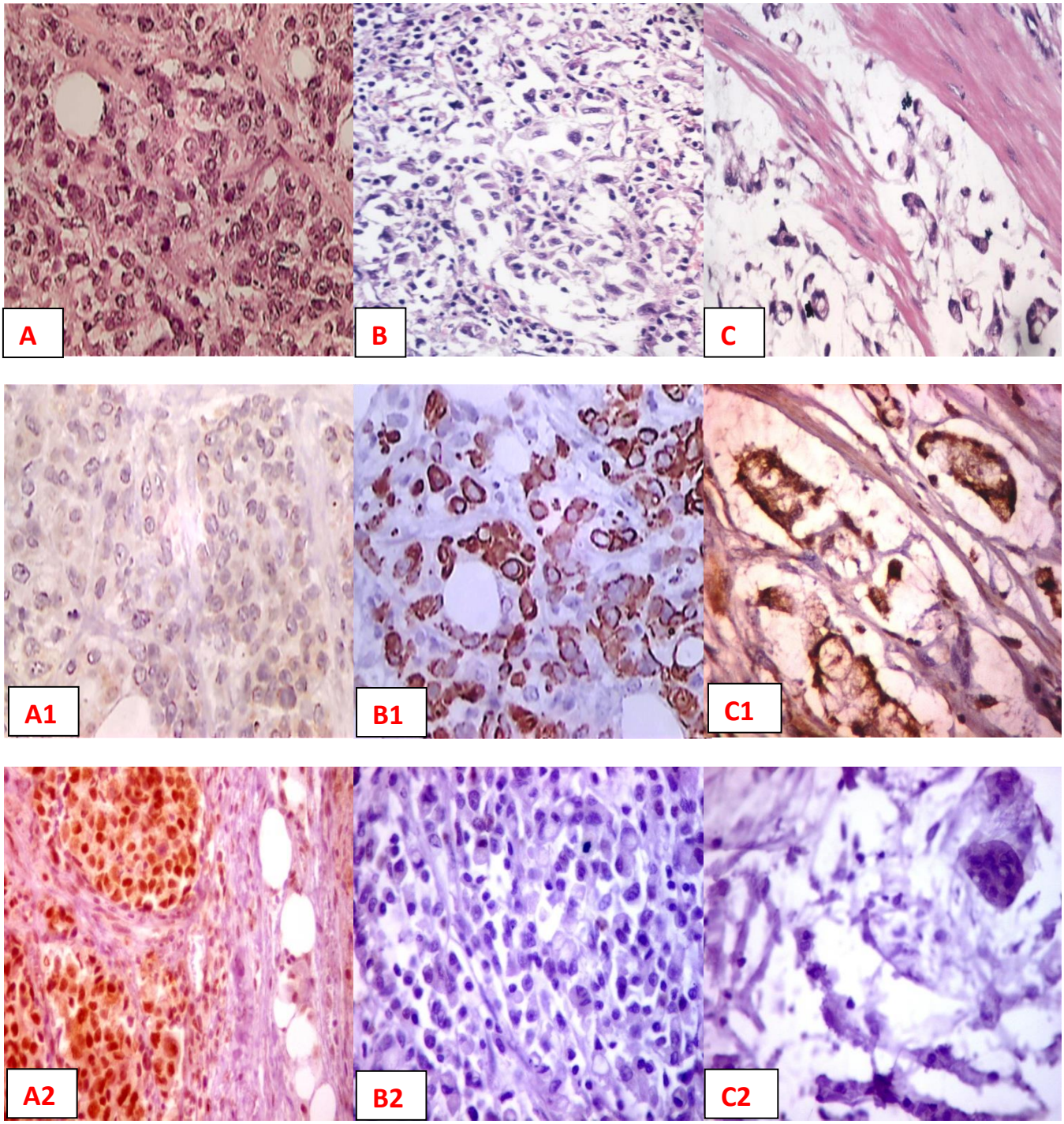

Figure 1: Representative micrograph from the research group

Table 2: Correlation between claudin-4 and ERCC1 immunohistochemistry expression and clinicopathological characteristics

	Claudin-4 low expression (N=71)	Claudin-4 high expression (N=84)	P	ERCC1− (N=64)	ERCC1+ (N=91)	P
Age						
<50	31(43.7%)	42(50%)	0.52	25(39.1%)	48(52.7%)	0.1
≥50	40(56.3%)	42(50%)		39(60.9%)	43(47.3%)	
Sex						
Male	51(71.8%)	48(57.1%)	0.07	39(60.9%)	60(65.9%)	0.61
Female	20(28.2%)	36(42.9%)		25(39.1%)	31(34.1%)	
Tumor size						
<2	9(12.7%)	19(22.6%)	0.28	12(18.8%)	16(17.6%)	0.78
2-5	45(63.4%)	47(56%)		36(56.3%)	56(61.5%)	
>5	17(23.9%)	18(21.4%)		16(25%)	19(20.9%)	
Site						
Fundus	19(26.8%)	29(34.5%)	0. 11	14(21.9%)	34(37.4%)	0.12
Body & antrum	50(70.4%)	47(56%)		45(70.3%)	52(57.1%)	
pylorus	2(2.8%)	8(9.5%)		5(7.8%)	5(5.5%)	
Shape						
Fungating	29(40.8%)	35(41.7%)	0.22	28(43.8%)	36(39.6%)	0.61
Infiltrating	18(25.4%)	30(35.7%)		21(32.8%)	27(29.7%)	
Ulcerating	24(33.8%)	19(22.6%)		15(23.4%)	28(30.8%)	
Histologic type						
Adenocarcinoma (Papillary& tubular)	45(63.4%)	56(66.7%)	0.88	24(37.5%)	77(84.6%)	**0.00**
Mucinous carcinoma	12(16.9%)	12(14.3%)		13(20.3)	11(12.1%)	
Signet ring carcinoma	6(8.5%)	5(6%)		9(14.1%)	2(2.2%)	
Undifferentiated carcinoma	8(11.3%)	11(13.1%)		18(28.1%)	1(1.1%)	
Grade						
Differentiated (well & moderate)	27(38%)	45(53.6%)	0.08	12(18.8%)	60(65.9%)	**0.00**
Less-differentiated (poor & undifferentiated)	44(62%)	39(46.4%)		52(81.3%)	31(34.1%)	
Lauren classification						
Intestinal	45(63.4%)	59(70.2%)	0.39	31(48.4%)	73(80.2%)	**0.00**
Diffuse	26(36.6%)	25(29.8%)		33(51.6%)	18(19.8%)	
Lymphatic invasion						
Yes	26(36.6%)	29(34.5%)	0.87	24(37.5%)	31(34.1%)	0.73
No	45(63.4%)	55(65.5%)		40(62.5%)	60(65.9%)	
Perineural invasion						
Yes	22(31%)	6(7.1%)	**0.00**	14(21.9%)	14(15.4%)	0.4
No	49(69%)	78(92.9%)		50(78.1%)	77(84.6%)	
T stage						
T1	0	7(8.3%)		0	7(7.7%)	
T2	29(40.8%)	54(64.3%)	**0.00**	31(48.4%)	52(57.1%)	**0.039**
T3	36(50.7%)	21(25%)		28(43.8%)	29(31.9%)	
T4a	6(8.5%)	2(2.4%)		5(7.8%)	3(3.3%)	
N stage						
N0	1(1.4%)	44(52.4%)		17(26.6%)	28(30.8%)	
N1	21(29.6%)	39(46.4%)	**0.00**	16(25%)	44(48.4%)	**0.001**
N2	39(54.9%)	1(1.2%)		23(35.9%)	17(18.7%)	
N3a	10(14.1%)	0		8(12.5%)	2(2.2%)	
TNM						
I (A,B)	0	31(36.9%)	**0.00**	10(15.6%)	21(23.1%)	**0.00**
II (A,B)	41(57.7%)	53(63.1%)		30(46.9%)	64(70.3%)	
III (A,B)	30(42.3%)	0		24(37.5%)	6(6.6%)	
With each other						
ERCC			**0.001**			
Negative	40(56.3%)	24(28.6%)				
Positive	31(43.7%)	60(71.4%)				
CHT protocols						
ELF	1(1.4%)	44(52.4%)		17(26.6%)	28(30.8%)	
Cispltin- 5fu	21(29.6%)	39(46.4%)		16(25%)	44(48.4%)	
CAP	39(54.9%)	1(1.2%)	**0.00**	23(35.9%)	17(18.7%)	
DCF	10(14.1%)	0		8(12.5%)	2(2.2%)	
Follow up data for 119 cases						
Disease relapse						
Absent	48(76.2%)	43(76.8%)	0.94	44(83%)	47(71.2%)	0.13
Present	15(23.8%	13(23.2%)		9(17%)	19(28.8%)	
Survival state						
Dead	39(61.9%)	23(41.1%)	**0.023**	31(58.5%)	31(47%)	0.27
Alive	24(38.1%)	33(58.9%)		22(41.5%)	35(53%)	
Disease free survival	12.7 m	18.9 m	**0.003**	16.1 m	13.7 m	0.85

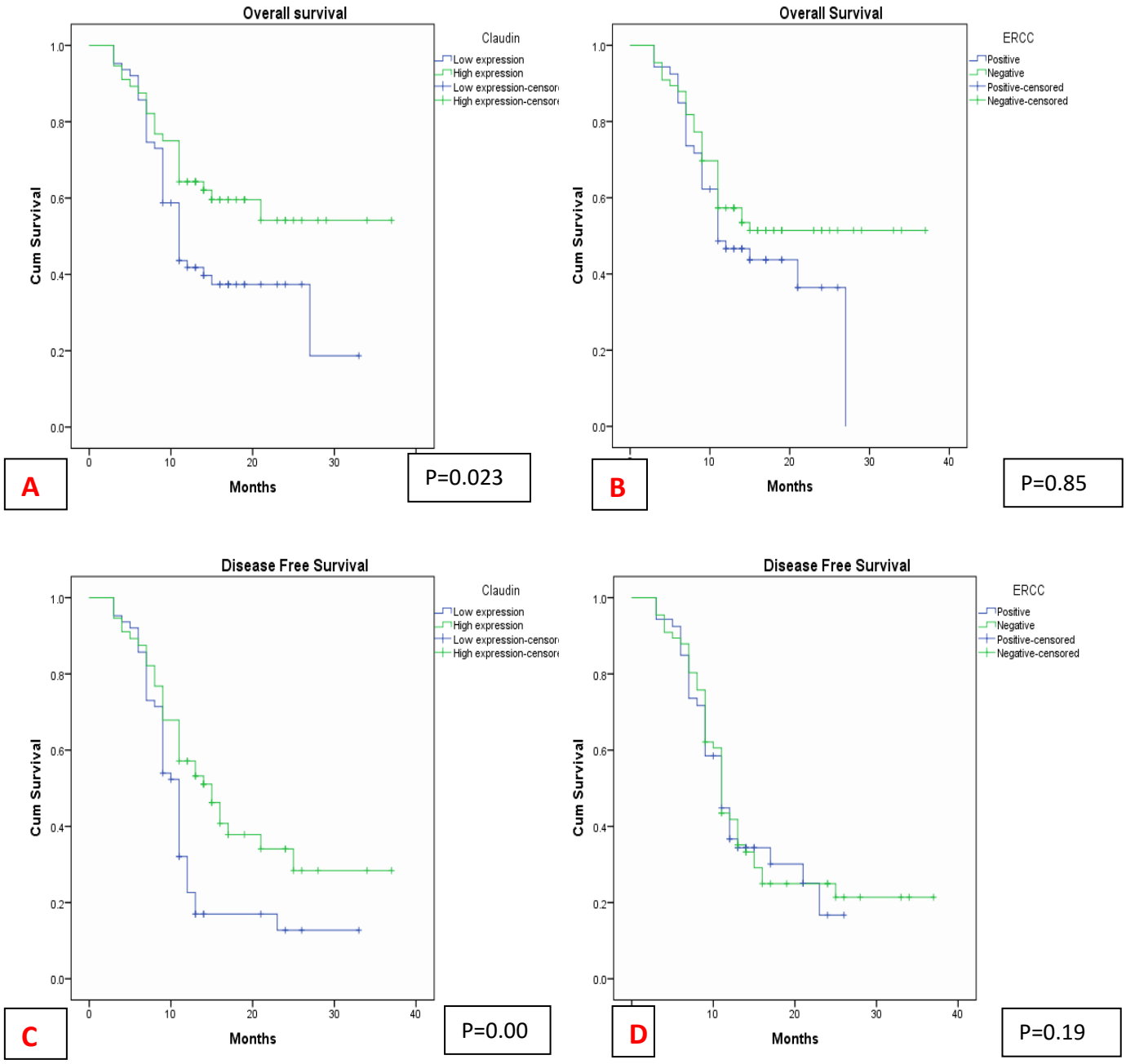

Figure 2: Kaplan-Meier curves for survival curves for gastric cancer patients based on the expression status of claudin-4 and ERCC1

Table 3: Univariate and multivariate analysis of clinicopathological factors affecting OAS & DFS

Variables	OAS		DFS	
	Univariate (Estimated mean)	Multivariate RR(95% CI)	Univariate (Estimated mean)	Multivariate RR(95% CI)
Age				
<50	31.8	15.4(6.5-36.5)	22.4	
≥50	11.5		9.9	5.3(3-9.3)
P value	**.00**	**.00**	**.00**	**.00**
Sex				
Male	20.3	1.3(0.6-2.9)	15.5	
Female	19.7		14.8	1.2(0.6-2.1)
P value	.15	.56	.39	.62
Tumor size				
<2	20.4	0.9(0.4-2.2)	18.3	
2-5	18.4	1.1(0.4-3)	13.6	.95(0.5-2)
>5	17.6		13.1	1(0.4-2.6)
P value	.9	.93	.76	.95
Histologic type				
Conventional	21.3		15.6	
Undifferentiated	17.1	0.8(0.1-6)	14.5	2.5(0.7-9.1)
Signet ring	15.3	0.6(0.1-5.5)	15.3	2.1(0.4-12)
Mucinous	17.1	2.6(0.3-18.4)	13.2	5(1.1-22.3)
P value	.51	.23	.7	.16
Grade				
Low	20.2		16.6	
High	20	0.9 (0.4-2.1)	14.6	1.5(0.8-2.8)
P value	.32	.75	.98	.24
Lauren classification				
Intestinal	20	0.8(0.1-4.9)	14.2	
Diffuse	21.5		18.6	0.2(0.05-0.8)
P value	.65	.8	12	.021
Lymphatic invasion				
Yes	18.4	0.8(0.4-1.7)	14.4	
No	21.2		15.9	0.7(0.4-1.2)
P value	.61	.58	.54	.15
Perineural invasion				
Yes	16.3	0.8(0.3-1.8)	11.9	
No	22.1		16.6	0.7(0.3-1.4)
P value	.39	.6	.14	.27
T stage				
T1	9.5	0.8(0.2-3)	8.7	
T2	20.1	2.8(0.6-11.7)	15.1	1(0.3-3.2)
T3	18.8	29.9(3.8-234.5);	15.9	1.9(0.5-6.7)
T4a	6.7	**.001**	6.7	10(1.7-59.7);**.012**
P value	**.001**	**.00**	**.009**	**.009**
N stage				
N0	20.4		20.4	
N1	25.8	0.7(0.3-1.8)	18	1.8(0.7-4.3)
N2	14.8	1.4(0.4-5.3)	10.4	3.4(1-11.5); **0.046**
N3a	8.9	5(0.99-25.2); **.051**	8.9	7(1.8-27.9); **0.006**
P value	**.004**	**.033**	**.00**	**.047**
ERCC				
Negative	23.2		16.1	
Positive	16	0.6(0.3-1.4)	13.7	2.2(1.1-4.3)
P value	.19	.27	.85	**.018**
Claudin-4				
Negative	16.6	1.2(0.4-3.6)	18.9	
Positive	24.5		12.7	0.9(0.4-2.1)
P value	**.023**	.76	**.003**	.8
Multivariate analysis running Cox regression model excluding T & N stage				
TNM stage				
I	17.9		17.2	
II	23.6	2.2(0.8-5.7)	16.4	3(1.2-7.1); **.014**
III	10.8	13.1(2.9-59); **.001**	9.8	9.5(2.8-32.2); **.00**
P value	**.00**	**.002**	**.002**	**.001**
CHT protocols				
ELF	20.4		20.4	1.8(0.7-4.3)
Cispltin- 5fu	25.8	0.7(0.3-1.8)	18	3.4(1-11.5); **0.046**
CAP	14.8	1.4(0.4-5.3)	10.4	7(1.8-27.9);
DCF	8.9	5(0.99-25.2); **.051**	8.9	**0.047**
P value	**.004**		**.00**	

Univariate and multivariate analysis of survival

Advanced T stage (P=0.001), increased number of LN metastasis (P=0.004), age above or equal to fifty (P=0.00), and low level of claudin-4 expression (P = 0.023) were all found to be significant prognostic indicators of poor overall survival in univariate analysis, with the exception of claudin-4, which lost its significance in multivariate analysis (Table 3). However, when utilizing univariate analysis to investigate DFS, it was discovered that age less than 50 (P=0.00), increased LN metastases (P=0.00), advanced T stage (P=0.009), and low claudin-4 expression (P = 0.003) were all indicators of reduced DFS. In addition to ERCC1 (P = 0.018), this was verified in multivariate analysis. The shift in importance of ERCC1 in connection to DFS from univariate to multivariate analysis could be explained by the suppressor variable effect. Using univariate and multivariate analysis, the TNM stage was a significant poor prognostic factor for both OS (P=0.00 and P=0.002) and DFS (P=0.002 and P=0.001) and types of chemotherapy OS (P=0.004 and P=0.051) and DFS (P=0.00 and P=0.047).

Discussion:

In the last decade, much has been learned about molecular alterations in GC but much remains to be learned about the most effective strategy for increasing survival rates. On a genomic level, cancer is defined by genome instability [19]. ERCC1 is required for the repair of damaged DNA and the maintenance of genetic information integrity. As a result, ERCC1 expression levels in cancers should roughly correlated with the tumor's inherent ability to repair DNA damage. The disruption of the DNA damage repair mechanism leads to an increase in genetic instability, which leads to faster tumor development and more malignant symptoms, as well as a bad prognosis [20].

The link between tight-junction-related proteins and gastric carcinogenesis and progression has been studied in a few different ways. Tight junctions play an important role in cell polarity, barrier function, and cell signaling pathways, as previously mentioned. Disruption of these tight junctions can result in cell polarity loss, as well as an aberrant influx of tumorigenic growth factors into epithelial cells via autocrine and paracrine signaling [21]. Tight-junction-associated proteins' specific involvement in gastric cancer, however, remains unknown.

Statistical significance was found between ERCC1 expression and Lauren categorization, histological grade and type, number of positive LN depth of invasion, and total TNM stage in this research. Positive ERCC1 was correlated to papillary and tubular adenocarcinoma, intestinal type, and a higher degree of differentiation. The ERCC1 protein is inherently unstable. ERCC1 and other variant genotypes have been linked to the risk of gastric cancer in a few studies [22]. The expression of ERCC1 may have a role in cancer growth.

Although no statistical significance was identified between survival outcome (except for DFS on multivariate analysis) and ERCC1 expression which might be explained in support of the hypothesis that patients receiving neoadjuvant treatment were not eliminated. Furthermore, because the data were incomplete and the follow-up duration was insufficient.

Almost all prior research looked on the relationship between ERCC1 expression and chemotherapy response. Even Wang and colleagues [20], who looked into the role of ERCC1 in prognosis, couldn't establish a statistical correlation with clinicopathological characteristics. Its effect in connection to platinum-based therapies has been examined in numerous studies, both in [GC] [11, 23] and other malignancies [13].

Reduced cell–cell or cell–matrix interactions are frequent in gastric cancers and may be more connected to metastatic susceptibility than the initial transformation processes. [25]. we discovered that increased claudin-4 expression was linked to lower T stage (T2, 40.8 percent), absent nodal metastases (52.4 percent), lower stage (63. percent, stage II), absence perineural invasion, and improved prognosis in this study. However, there was no significant connection between histological type and grade in our research. Lee et al. [26], for example, found that decreased levels of claudin-4 expression were linked to loss of differentiation.

Furthermore, we discovered that the group expressing high levels of claudin-4 had a considerably superior survival rate (33/57alive), as well as an increase in DFS and OS, indicating that it may be utilized as a prognostic marker. On univariate and multivariate analyses, this was verified. The question of claudin-4's predictive value remains unanswered. In 146 patients, Resnicket al. [28] found that high claudin-4 expression was linked with a poor prognosis, but Soini et al. [29] and Zhu et al. [27] found that claudin-4 was not connected with OS.

Both proteins' IHC expression revealed a strong connection among each other. The existence of numerous hits, inactivating genes regulating DNA repair, and cell-cell contacts, according to the general principles of carcinogenesis, clearly demonstrates the complex mutational interactions involved in the process of carcinogenesis [30].

The conventional variables utilized in deciding treatment methods have been histology characteristics and TNM categorization. In both univariate and multivariate analyses, these variables revealed a significant association with survival. Biological aggressiveness, prognostic correlations, and therapeutic responsiveness may vary across lesions with identical shapes. Despite the fact that low ERCC1 expression was thought to be a poor prognostic factor, patients responded better to chemotherapy and were more likely to benefit from it. All of this highlights the need for further prospective research to better understand the molecular changes involved in gastric cancer pathogenesis and to identify early indicators of cell transformation. As a result, we may be able to assess an individual's cancer risk and, as a result, devise a treatment strategy tailored to each patient.

Recently, Patients with ERCC1-high gastric carcinoma had a lower cumulative incidence function estimate of cancer-related death [3.37; 95 percent (CI)=0.89-8.75] than those with ERCC1-low gastric carcinoma (17.12; 95 percent CI=12.24-22.69; p-value by Gray's test=0.0012) than those with ERCC1-low gastric carcinoma (17.12; 95 percent CI=12.24-22.69; p-value The adjusted proportional sub-distribution hazard ratio for cancer-related death in patients with ERCC1-high tumors was 0.272 (95 percent CI=0.084-0.878; p=0.0295) [31].

In contrast with these results, several studies demonstrate that ERCC1 overexpression correlates with better survival in curatively resected gastric cancer patients treated with adjuvant platinum-based regimens [32-33]. In addition, in patients treated with platinum-based regimens, low ERCC1 expression by IHC was associated with a higher response rate and survival.

Conclusion:

When ERCC1 expression is negative and Claudin-4 expression is low, may play a role in gastric carcinogenesis and development by disrupting cell tight junction and genomic stability. Furthermore, a high level of claudin-4 expression was linked to a long lifetime. At the same time, a thorough understanding of the role of the ERCC-1 gene in the incidence, progression, and metastasis of GC is important for early diagnosis and treatment of early tumor.

We recommend for further study to assess the relation with different chemotherapy protocols for more selection of the better one with benefit survival.

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