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RESEARCH ARTICLE

The prognostic significance of minichromosome maintenance protein 2 (MCM2) and Trans-glutaminase 2 (TGM2) in Endometrial carcinoma: immunohistochemical and serological study

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ABSTRACT

Background: Endometrial carcinoma (EC) is a common gynecologic malignancy, with rising incidence. Unfortunately, it has poor prognosis with declining 5-years survival rate. Discovering target biomarkers for EC is essential to identify novel therapeutic options. Minichromosome maintenance 2 (MCM2) and Trans-glutaminase 2 (TGM2) are of these studied biological biomarkers. **Patients and Methods**: This prospective study was carried out on 60 patients diagnosed with EC. All patients were treated and followed up at Tanta University Hospitals. Immunohistochemistry was performed using MCM2 and TGM2 primary antibodies, as well as serum TGM2 levels were measured and compared with other clinicopathological parameters. **Results**: MCM2 and TGM2 showed high expressions in 53.3% & 48.3% of cases respectively. Their expressions were associated with myometrial, cervical, lympho-vascular invasion, and higher tumor grade (P <0.001, P = 0.022, P = 0.011, P = 0.017 respectively) for MCM2 and (P=0.032, P=0.012, P=0.001, P=0.035 respectively) for TGM2. High MCM2 expression was associated with advanced FIGO stage (P = 0.001). High serum TGM2 levels were associated with serous type EC compared to endometrioid type (P=0.02) and with the presence of myometrial invasion (P<0.001). The 3-years disease free survival rate was 78.33%. Univariate analysis revealed that FIGO staging, myometrial, lymphovascular invasion, MCM2 and TGM2 expressions, together with TGM2 serum levels had statistically significant relation to DFS. **Conclusions**: High MCM2 and TGM2 expressions as well as high serum TGM2 levels are significantly associated with unfavorable prognostic factors and poor survival outcome in EC patients.

Keywords: Endometrial carcinoma, MCM2, TGM2

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INTRODUCTION

Endometrial carcinoma (EC) is the fourth most common female malignant neoplasm. It is considered the commonest malignant gynecologic tumor in the developed countries and the second common in the developing ones (Siegel et al., 2019). In Egypt, EC accounts for about 22.83% of all female gynecologic malignancies as documented by Cancer Pathology Registry, National Cancer Institute. In the last years, the incidence of EC has increased, and its 5-year survival rate has decreased. So, studying the basic of EC is mandatory and attaining more consideration (Mokhtar et al., 2016).

Endometrial carcinoma is known to be a malignancy of the postmenopausal women; however, about 14% of cases are detected in premenopausal females; of whom 5% are less than 40 years. Exposure to estrogen (either endogenous or exogenous), diabetes, obesity, early menarche, late-onset menopause, and null parity are the major risk factors for the development of EC (Morice et al., 2016).

Endometrial carcinoma is classified into two types: type I (estrogen-dependent tumors), which accounts for 80%- 85% of cases, including endometrioid endometrial carcinoma (EEC), and type II (estrogen-independent tumors), including serous endometrial carcinoma (SEC) and clear cell carcinoma (del Carmen et al.,

2012). Type II endometrial carcinomas demonstrate a greater incidence of myometrial and lymphovascular invasion, with more aggressive clinical outcomes (Soslow et al., 2007).

Endometrial carcinoma is liable to recurrence and metastasis, leading to unfavorable prognosis in many cases (Brooks et al., 2019). Thus, discovering novel targets aids in the diagnosis, prognosis, management, and provides specific therapeutic strategies, which will be of great benefit for EC patients.

Tumors develop greatly due to disorders of the cell cycle. Cell cycle regulation is complex and includes several proteins and genes. The minichromosome maintenance (MCM) proteins are necessary for DNA duplication and cell cycle initiation and progression (Gonzalez et al., 2005). Minichromosome maintenance protein 2 (MCM2) is one of the six MCM proteins (MCM 2–7). MCM2 regulates and stabilizes the pre-replication complexes. This makes MCM2 an appropriate indicator of cell proliferation (Kang et al., 2014).

MCM2 expression has been investigated in various malignancies such as gastrointestinal, renal, and bladder cancer (Guo et al., 2018). Deregulation of MCM2 protein is associated with tumor initiation, progression, and malignant transformation. Therefore, MCM2 could be associated with poor clinicopathological features such as higher grades, advanced stages, and poor prognosis (Abe et al., 2015).

Trans-glutaminase 2 (TGM2) belongs to transglutaminase family of enzymes which get activated in the presence of Ca2+ and catalyze Ca2+-dependent protein cross linking via amide bonds formation. TGM2 is expressed in nearly all cell compartments including the cytoplasm, mitochondria, recycling endosomes, and nucleus. It also exists on the cell surface and becomes secreted in the extracellular matrix (ECM) (Belkin, 2011). TGM2 can modify the ECM to support the integrin-dependent extracellular matrix binding and migration of malignant cells (Dominika and Agnieszka, 2019). Furthermore, TGM2 induces epithelial-to-mesenchymal transition (EMT) which facilitates tumor metastasis (Shinde et al., 2020).

Various studies show that malignant cells express high levels of TGM2, and that the overexpression of TGM2 is related to aggressive tumor behavior and drug resistance in various cancers. Also, TGM2 levels are specifically elevated in cancer stem cell, which is necessary for their survival, invasion, and migration (Eckert et al., 2015).

Although MCM2 and TGM2 expression levels have been studied in different cancers, few studies were conducted to evaluate their possible prognostic effect in EC. The current study aims to explore the immunohistochemical expression of MCM2 and TGM2 in endometrial carcinoma, as well as TGM2 serum level to investigate the possible relation between those markers with various clinicopathological features in EC patients, and their relation to survival.

PATIENTS AND METHODS

This prospective study included 60 patients diagnosed with endometrial carcinoma during the period from October 2018 to October 2022. This study was carried out in Pathology, Clinical Oncology, and Clinical Pathology Departments, Faculty of Medicine, Tanta University. The study was approved by the local research ethical committee, Institutional Review Board (IRB) for human studies, Faculty of Medicine, Tanta University (Approval Code 35952). This study conforms to provisions of the Declaration of Helsinki.

All patients underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy. Treatment and follow-up of patients were done at Clinical Oncology Department, Faculty of Medicine, Tanta University. Patients were evaluated every 3 months during the first year and every 6 months after the first year. All Patients received adjuvant radiotherapy ± chemotherapy (following clinical stage and current treatment guidelines). Patients were excluded from the study if they had metastatic disease, another malignancy, incomplete surgical staging, and serious illness (like myocardial infarction) within 3 months.

Radiotherapy was indicated in patients with stage III, II and high-risk stage I (high risk criteria

like older age, myometrial invasion > half thickness, lymphovascular invasion, and grade II or III). The adjuvant radiotherapy was given 6 weeks after surgery in the form of External Beam Three D Conformal Radiotherapy (3DCRT) Technique with Varian Unique LINAC, dose of (45 Gy in 1.8 Gy fractions, 5 weeks). Clinical Target Volume TV) coverage was insured in pelvic tissues at risk and pelvic lymph nodes. Adjuvant chemotherapy was indicated in patients with stage III and high-risk stage II in the form of carboplatin AUC=6 repeated every three weeks and weekly paclitaxel 80 mg/m2 IV for 6 cycles.

The surgical specimens were referred to the Pathology department, Faculty of Medicine, Tanta University. Formalin-fixed paraffin-embedded (FFPE) blocks were prepared. Hematoxylin and Eosin (H&E) stained sections were examined, to confirm histopathological diagnosis. Patient's data were recorded and analyzed including age, histopathological type, tumor grading, presence of lymphovascular invasion (LV), myometrial and cervical invasion. Tumor stage and grade were classified according to the International Federation of Gynecology and Obstetrics (FIGO) criteria (Soslow et al., 2019). Disease-free survival (DFS) was recorded and was defined as the interval between the date of start of treatment and either recurrence or metastasis of the tumor.

Immunohistochemical staining

After de-waxing, inactivation of endogenous peroxidase activity, and blocking the cross-reactivity with normal serum, immunohistochemical staining was done. Overnight incubation in a humidity chamber was done with a rabbit monoclonal anti-MCM2 antibody (Clone EPR3727) (Abcam, Egypt), dilution 1: 50 and a mouse monoclonal anti-TGM2 antibody (Clone CUB7402) (Abcam, Egypt), dilution 1:50. This was followed by washing in phosphate-buffered saline (PBS), then covering with 4–5 drops of Ultra Vision biotinylated goat anti-polyvalent secondary antibody, incubated at room temperature for 10 min, then washed in PBS. Finally, counterstaining with Meyer's Hematoxylin solution was performed, and slides were covered.

Immuno-stained slides were examined under magnification x400. MCM2 showed nuclear staining. Labeling index was expressed as the percentage of positively stained tumor cells based on a count of at least 1,000 cancer cells. A labeling index more than 20% was considered a cutoff point. MCM2 immunoexpression was classified as low expression (less than 20%) and high expression (more than 20%) (Mehdi et al., 2016).

For TGM2 cytoplasmic and/or membranous staining of cells was considered positive. TGM2 immunostaining was assessed according to the intensity and percentage of positive stained tumor cells. The extent of positive tumor cells was classified into 5 categories; 0 (<5%), 1 (5%-25%), 2 (26%-50%), 3 (51%-75%) and 4 (>75%) cells stained. Staining intensity was graded from 0 to 3 for negative, weak, moderate, and strong staining, respectively. Final score was calculated by summation of both scores, with (0-3) scores considered as low expression, and scores of (4-7) as high expression (Erdem et al., 2015).

Blood samples collection

Whole peripheral blood samples were collected from all participants by standard venipuncture under complete aseptic precautions in VACUETTE® Blood Collection plain Tubes. After centrifugation, serum was separated and stored at −80 °C until the time of laboratory assay of TGM2.

Serum TGM2 immunoassay

Enzyme linked immunosorbent assay was used to measure serum TGM2 levels (Human TGM2, Invitrogen ELISA Kit, Catalog Number EH462RB; Thermo Fisher Scientific, USA) in full compliance with the manufacturer's instructions. Samples were diluted before assay 1:3 with the assay diluent provided with the kits. Serial dilution was made from a standard stock of 200 ng/ml in order to create the standard curve. Samples analyzed using a Tecan Spectra II Microplate Reader (Switzerland) at optical density (O.D) 450 nm. The concentrations of samples were calculated from a logit-log standard curve using a curve-fitting program, curve expert 1.4.

Statistical analysis

Statistical Package for Social Science (version 24) was used to perform the statistical analysis.

Data were expressed as frequencies for categorical variables, and mean ±SD or median for the continuous variables. Chi-square ($\chi 2$) was used as the test of significance to compare categorical variables. Monte Carlo test was used when appropriate. P-value ≤ 0.05 was considered statistically significant. Survival Kaplan Meier analysis has been used to compare DFS in different expression of the studied markers.

RESULTS

The present study included 60 cases of endometrial adenocarcinoma. Their age ranged from 39 to 78 years with a mean of 60 ± 9.75 years. About 75% of the cases were endometrioid endometrial carcinoma (EEC), while 25% were serous endometrial carcinoma (SEC). Regarding FIGO grade, 15% of the cases were Grade 1, 45% were Grade II, and 40% were Grade III. Cases were classified according to FIGO stage as 41.7% were Stage I, 53.3 % were Stage II and 5% were Stage III. Myometrial invasion > half thickness was documented in 43.3 % of the cases. Cervical involvement was seen in 36.7% of the cases and 28.3% of cases showed lymphovascular invasion. The clinicopathological characteristics of the studied cases are summarized in Table 1.

MCM2 immunohistochemical expression results and its relation to clinicopathological parameters

MCM2 showed high expression in 53.3% of cases whereas 46.7% of cases were of low expression (Figure 1). Grade III cases displayed the highest rate of MCM2 overexpression (66.7%) followed by Grade II and then I with statistically significant difference (P = 0.017). There was a statistically significant relation between MCM2 expression and FIGO stage (P = 0.001) with 100% of stage III cases were of high expression.

There were significant relations between high MCM2 expression and myometrial invasion (P <0.001), cervical and lymphovascular invasion (P = 0.022 and P = 0.011 respectively). No significant association was detected regarding age and histologic type (P=0.330 and 0.084), respectively; however, MCM2 expression in SEC (73.3%) was higher than EEC (46.7%) but did not reach the statistically significant level. Relation of MCM2 expression with various clinicopathological parameters is summarized in Table 2.

TGM2 immunohistochemical expression results and its relation to clinicopathological parameters

High TGM2 expression was detected in 48.3% of cases whereas 51.7% of cases showed low expression (Figure 2). There was a statistically significant relation between TGM2 expression and tumor grade (P = 0.001) with 75% of grade III cases showed high expression.

Significant relations between high TGM2 expression and myometrial invasion (P =0.035), cervical and lymphovascular invasion (P = 0.032 and P = 0.012 respectively) were detected. No significant association was detected regarding age, histologic type and FIGO stage (P=0.821, 0.296 and 0.118), respectively. TGM2 expression in SEC (60 %) was higher than EEC (44.4%) but did not reach the statistically significant level. Relation of TGM2 expression with various clinicopathological parameters is summarized in Table 3.

TGM2 serum level results and their relation to the clinicopathological parameters

Serum level of TGM2 in EC patients ranged from 33-193 ng/ml with mean value 93.2± 44ng/ml. Cases were divided into high and low groups according to the median level > 77 and < 77 ng/ml, respectively. High TGM2 serum levels were observed in 45% of cases whereas 55% of cases showed low TGM2 levels. TGM2 serum level was significantly higher in serous type EC compared to endometrioid type (P = 0.02). A statistically significant relations was observed between high TGM2 serum level and myometrial invasion (P <0.001). However, TGM2 serum level was higher in cases with cervical and lymphovascular invasion, but it did not reach the statistically significant level (P=0.11 and P=0.08) respectively.

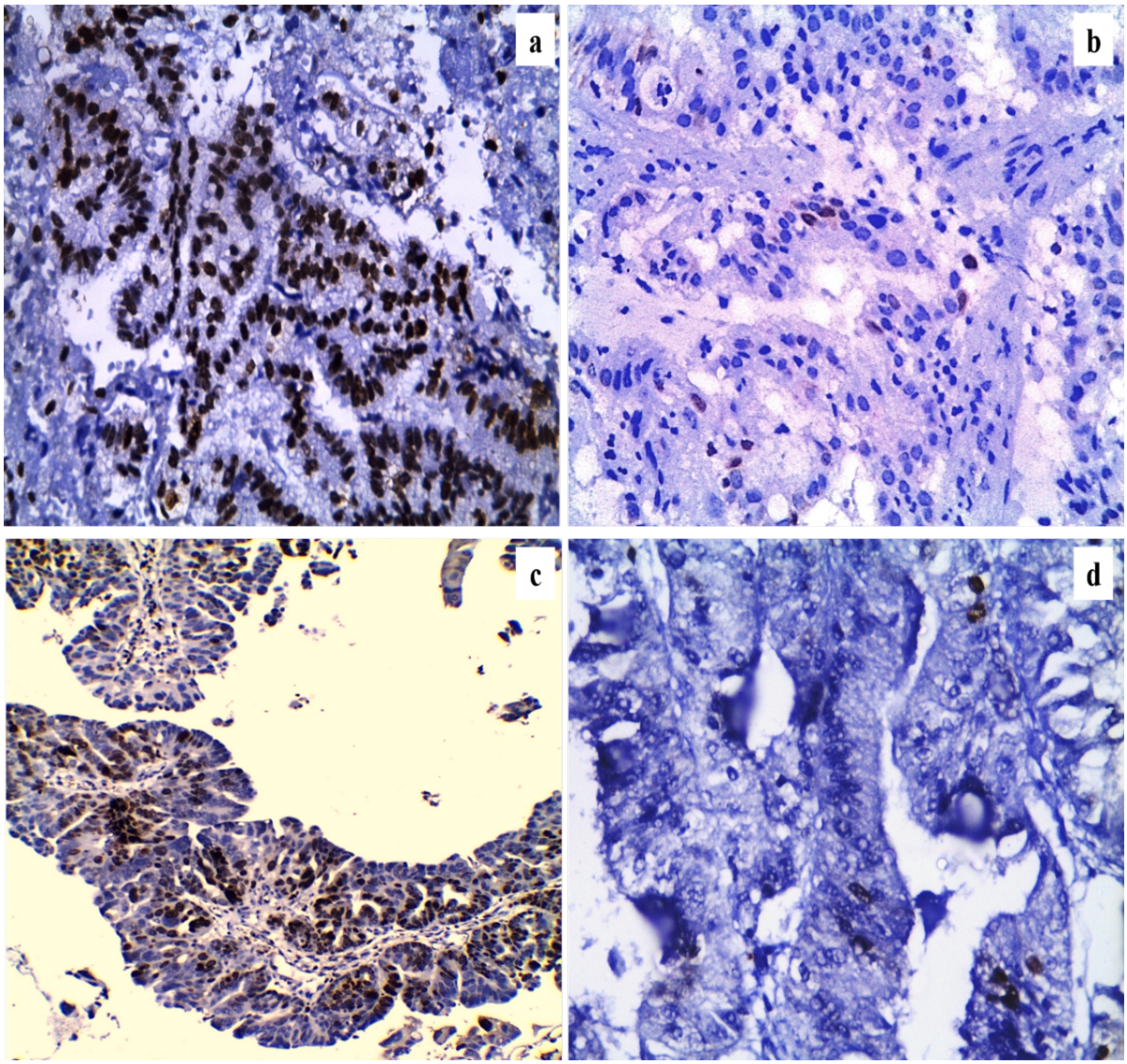

Figure 1. Minichromosome maintenance 2 (MCM2) immunohistochemical expression in endometrial carcinoma. Endometrioid endometrial carcinoma showing high MCM2 expression [x200] (a), Endometrioid endometrial carcinoma showing low MCM2 expression [x200] (b), Serous endometrial carcinoma showing high MCM2 expression [x100] (c), Serous endometrial carcinoma showing low MCM2 expression [x400] (d)

No significant difference was also detected regarding age, histological grade, and FIGO stage (P=1.00, 0.11 and 0.20), respectively. Relation of TGM2 serum levels with various clinicopathological parameters is summarized in Table 4.

In the current study, relapse occurred in 13 cases (21.67%) and the 3-years DFS rate was 78.33%. Table 5 shows the relation between the studied clinicopathological parameters and the progression of the disease. The FIGO staging, myometrial invasion, lymphovascular invasion, MCM2 and TGM2 immunohistochemical expressions, as well as TGM2 serum levels had statistically significant relation to DFS.

On performing univariate analysis, The FIGO staging, myometrial invasion, lymphovascular invasion, MCM2 and TGM2 immunohistochemical expressions, together with TGM2 serum levels had statistically significant relation to DFS as shown in Table 6 and Figure 3.

DISCUSSION

Endometrial carcinoma (EC) is a common gynecologic malignancy, with rising incidence. Unfortunately, its prognosis is poor with declining 5-year survival rate (Morice et al., 2016).

Table 1. Clinicopathological characteristics of the studied cases

Clinicopathological characteristics	Cases (No=60)	%
Age (Years)		
≤ 60	26	43.3
>60	34	56.7
Histological types		
Endometrioid endometrial carcinoma	45	75
Serous endometrial carcinoma	15	25
Histopathological grade		
I	9	15
II	27	45
III	24	40
FIGO stage		
I	25	41.7
II	32	53.3
III	3	5
Myometrial invasion		
< Half myometrial thickness	34	56.7
> Half myometrial thickness	26	43.3
Cervical invasion		
Absent	38	63.3
Present	22	36.7
Lymphovascular invasion		
Negative	43	71.7
Positive	17	28.3

Table 2. Minichromosome maintenance 2 (MCM2) immunohistochemical expression in relation to various clinicopathological parameters

Clinicopathological Parameters	Cases (No)	High MCM2 expression (n = 32, 53.3%)	Low MCM2 expression (n = 28, 46.7%)	P-value
Age (Years)				
≤ 60	26	12 (46.2%)	14 (53.8%)	0.330
>60	34	20 (58.8%)	14 (41.2%)	
Histological types				
Endometrioid endometrial carcinoma	45	21 (46.7%)	24 (53.3%)	0.084
Serous endometrial carcinoma	15	11 (73.3%)	4 (26.7%)	
Histopathological grade				
I	9	1 (11.1%)	8 (88.9%)	
II	27	15 (55.6%)	12 (44.4%)	**0.017***
III	24	16 (66.7%)	8 (33.3%)	
FIGO stage				
I	25	7 (28%)	18 (72%)	
II	32	22 (68.8%)	10 (31.2%)	**0.001***
III	3	3 (100%)	0 (0%)	
Myometrial invasion				
< Half myometrial thickness	34	11 (32.4%)	23 (67.6%)	**<0.001***
> Half myometrial thickness	26	21 (80.8%)	5 (19.2%)	
Cervical invasion				
Absent	38	16 (42.1%)	22 (57.9)	**0.022***
Present	22	16 (72.7%)	6 (27.3)	
Lymphovascular invasion				
Negative	43	18 (41.9%)	25 (58.1%)	**0.011***
Positive	17	14 (82.4%)	3 (17.6%)	

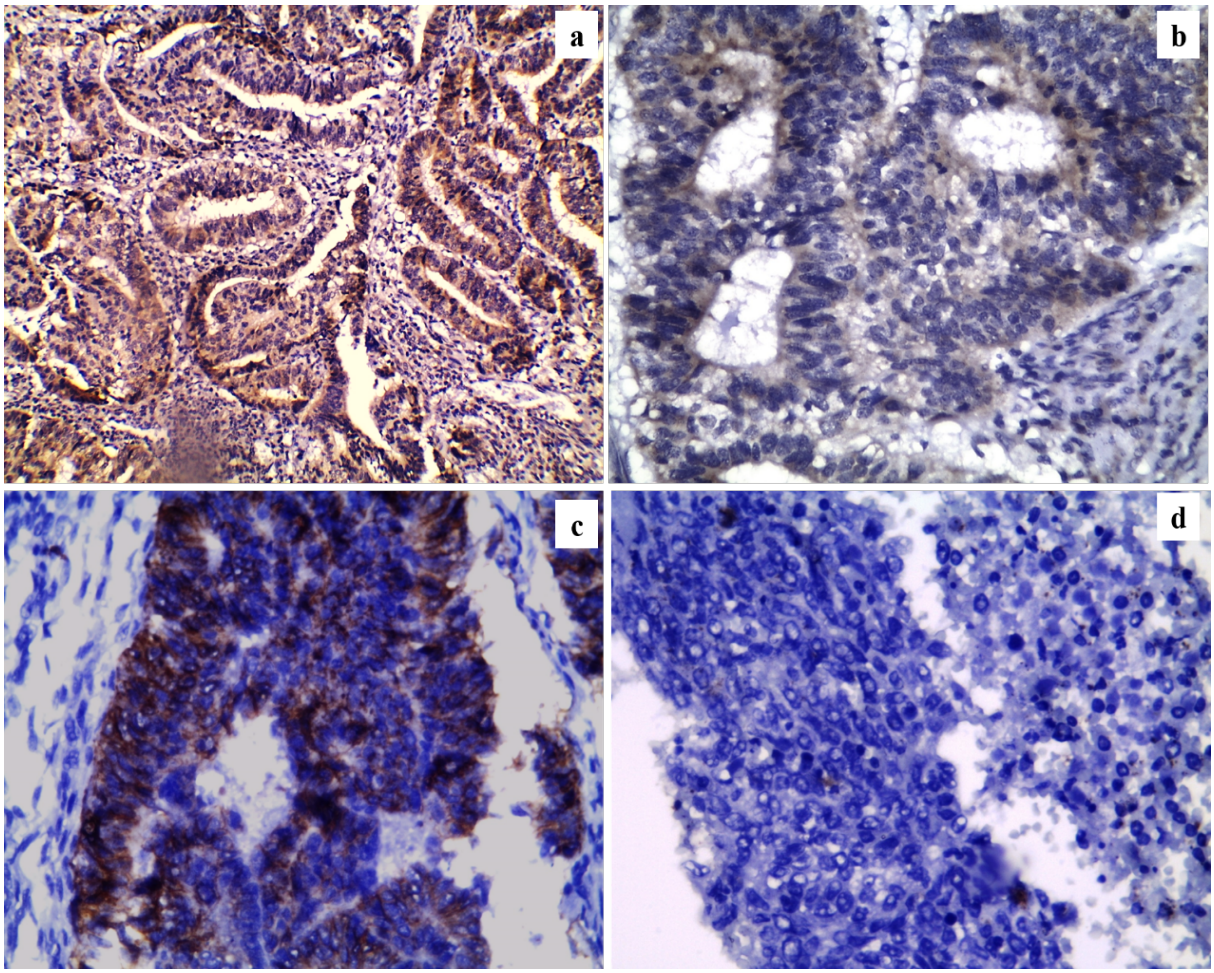

Figure 2. Trans-glutaminase 2 (TGM2) immunohistochemical expression in endometrial carcinoma. Endometrioid endometrial carcinoma showing high TGM2 expression [x100] (a), Endometrioid endometrial carcinoma showing low TGM2 expression [x400] (b), Serous endometrial carcinoma showing high TGM2 expression [x400] (c), Serous endometrial carcinoma showing negative TGM2 expression [x400] (d)

Discovering target biomarkers including mutated genes and molecules in EC is an important step to identify novel treatment options. There are many promising biomarkers described in this setting. Minichromosome maintenance 2 (MCM2) and Trans-glutaminase 2 (TGM2) are of these studied biological biomarkers (Dominika and Agnieszka, 2019).

In the present study, we studied the immunohistochemical expression of MCM2 and TGM2 in endometrial carcinoma, as well as TGM2 serum level to investigate the possible relation between those markers with various clinicopathological features in EC patients, and their relation to survival.

In the current study, immunohistochemical expression of MCM2 was high in 53.3% of cases. There was a significant relation between MCM2 expression, histopathological grade and FIGO stage; being higher in advanced grade and stage. MCM2 expression was significantly higher with myometrial invasion, cervical and lymphovascular invasion.

Our study matched those of Guo et al., 2018 who found that MCM2 expression showed significant difference among normal endometrium, endometrial hyperplasia, and endometrial carcinoma tissues; being higher in endometrial carcinoma specimens. They stated also that high MCM2 expression was related to the histopathological grade and the presence of myometrial invasion.

In this work, MCM2 expression in SEC was higher than EEC but did not reach the statistically significant level. Hiramatsu et al., 2016 also reported higher MCM2 immunohistochemical expression in ovarian and endometrial SEC than in EEC.

Table 3. Trans-glutaminase 2 (TGM2) immunohistochemical expression in relation to various clinicopathological parameters

Clinicopathological Parameters	Cases (No)	High TGM2 expression (n = 29, 48.3%)	Low TGM2 expression (n= 31, 51.7 %)	P-value
Age (Years)				
≤ 60	26	13 (50%)	13 (50%)	0.821
>60	34	16 (47.1%)	18 (52.9%)	
Histological types				
Endometrioid endometrial carcinoma	45	20 (44.4%)	25 (55.6%)	0.296
Serous endometrial carcinoma	15	9 (60%)	6 (40%)	
Histopathological grade				
I	9	5 (55.6%)	4 (44.4%)	**0.001***
II	27	6 (22.2%)	21 (77.8%)	
III	24	18 (75%)	6 (25%)	
FIGO stage				
I	25	16 (64%)	9 (36%)	
II	32	12 (37.5%)	20 (62.5%)	0.118
III	3	1 (33.3%)	2 (66.7%)	
Myometrial invasion				
< Half myometrial thickness	34	12 (35.3%)	22 (64.7%)	**0.035***
> Half myometrial thickness	26	17 (65.4%)	9 (34.6%)	
Cervical invasion				
Absent	38	14 (36.8%)	24 (63.2%)	
Present	22	15 (68.2%)	7 (31.8%)	**0.032***
Lymphovascular invasion				
Negative	43	17 (39.5%)	26 (60.5%)	**0.012***
Positive	17	12 (70.6%)	5 (29.4%)	

They also reported that the knockdown of MCM2 by silencing RNAs (siRNAs) significantly decreased the proliferation rate of SEC.

In contrast to our study, Kato et al., 2003 reported that MCM2 expression was significantly lower in endometrial carcinomas than that in the non-malignant endometrium. They reported a significantly higher MCM2 expression in the proliferative endometrial glands than in the secretory phase. They found significant relation between MCM2 expression in endometrial hyperplasia cases. Their findings suggested that MCM2 expression may reflect the cellular proliferation capacity in normal and hyperplastic endometrium, with probable aberrant replication-licensing system in EC.

In the current study, the 3-years DFS rate was 78.33%. MCM2 immunohistochemical expression had a statistically significant relation to DFS; being shorter with high MCM2 expression. Similar data were reported by Guo et al., 2018 who found that the 3-years survival rate was significantly lower with high MCM2 expression levels. Therefore, MCM2 can be regarded as a prognosticator in EC.

MCM2 expression have been studied in various malignancies rather than endometrial carcinoma. Gakiopoulou et al., 2007 found that MCM2 expression was higher in ovarian carcinomas compared to tumors with low malignant potential. They reported that MCM2 was significantly higher with advanced histopathological grade and stage. They also stated that high MCM2 expression indicated poor patient outcome in ovarian carcinoma.

Shetty et al., 2005 reported that high MCM2 expression in invasive breast carcinoma was related to larger tumor size, tumor histopathological grade, and the Nottingham Prognostic Index (NPI), but not associated with patient age, lymph node involvement, and vascular invasion.

Guzińska-Ustymowicz et al., 2008 stated that MCM2 expression in colorectal carcinoma (CRC) was significantly higher than its expression in normal colonic glands and adenomas.

Table 4. Trans-glutaminase 2 (TGM2) serum levels in relation to various clinicopathological parameters

Clinicopathological Parameters	Cases (No)	High serum TGM2 level (n=27, 45%)	Low serum TGM2 level (n=33, 55%)	P-value
Age (Years)				
≤ 60	26	12 (46.2%)	14 (53.8%)	1.00
>60	34	15 (44.1%)	19 (55.9%)	
Histological types				
Endometrioid endometrial carcinoma	45	16 (35.6%)	29 (64.4%)	**0.02***
Serous endometrial carcinoma	15	11 (73.3%)	4 (26.7%)	
Histopathological grade				
I	9	5 (55.6%)	4 (44.4%)	0.11
II	27	8 (29.6%)	19 (70.4%)	
III	24	14 (58.3%)	10 (41.7%)	
FIGO stage				
I	25	11 (44%)	14 (56%)	
II	32	13 (40.6%)	19 (59.4%)	0.20
III	3	3 (100%)	0 (0%)	
Myometrial invasion				
< Half myometrial thickness	34	8 (23.5%)	26 (76.5%)	**<0.001***
> Half myometrial thickness	26	19 (73.1%)	7 (26.9%)	
Cervical invasion				
Absent	38	14 (36.8%)	24 (63.2%)	0.11
Present	22	13 (59.1%)	9 (40.9%)	
Lymphovascular invasion				
Negative	43	16 (37.2%)	27 (62.8%)	0.08
Positive	17	11 (64.7%)	6 (35.3%)	

They reported that MCM2 high expression was associated with lymph node metastases, but not with patient age or tumor location.

The clinical impact of MCM2 in non-small cell lung carcinoma (NSCLC) was studied by Sakai et al., 2022 who stated that high MCM2 expression was associated with adenocarcinoma subtype, lymph nodal, and distant metastasis. They stated that high MCM2 was an independent factor for an unfavorable outcome with poor overall and progression free survivals. They also concluded that MCM2 could be used as a potential therapeutic target for NSCLC patients.

Ananthanarayanan et al., 2006 reported high MCM2 expression in prostatic carcinoma, but low and limited to the basal cell layer in non-malignant prostatic glands. They also reported that high MCM2 expression was associated with shorter disease-free survival.

Giaginis et al., 2010 stated that MCM2 was a more optimal tumor proliferative marker than the conventional PCNA and Ki-67 owing to its higher specificity and sensitivity to distinguish between benign, dysplastic, and malignant lesions. Boyd et al., 2008 revealed significant differences in MCM2 expression between benign or dysplastic nevi and malignant melanoma. Therefore, MCM2 expression may provide an important tool to distinguish benign and malignant melanocytic tumors. All the previous results suggest that MCM2 may be used as prognostic and diagnostic tool of a great value in several types of human malignancies.

Several serum markers were implicated in endometrial carcinoma; however, none has attained a high accuracy as a screening tool. Among these markers is Trans-glutaminase 2 (TMG2). TGM2 contributes in cell cycle regulation, apoptosis, wound healing, and cancer development. TGM2 is implicated in the etiology of many cancer types including pulmonary, breast, ovarian, pancreatic, prostatic, colonic carcinoma, and leukemia (Eckert, 2019).

Table 5. Relation between the clinicopathological parameters with 3-years disease free survival (DFS) and relapse

Clinicopathological Parameters	Cases (No)	3-years DFS (n= 47, 78.33%)	Relapse (n= 13, 21.67%)	P-value
Age (Years)				
≤ 60	26	20 (76.9%)	6 (23.1%)	1.00
>60	34	27 (79.4%)	7 (20.6%)	
Histological types				
Endometrioid endometrial carcinoma	45	37 (82.2%)	8 (17.8%)	0.28
Serous endometrial carcinoma	15	10 (66.7%)	5 (33.3%)	
Histopathological grade				
I	9	8 (88.9%)	1 (11.1%)	0.53
II	27	22 (81.5%)	5 (18.5%)	
III	24	17 (70.8%)	7 (29.2%)	
FIGO stage				
I	25	23 (92%)	2 (8%)	**0.03***
II	32	23 (71.9%)	9 (28.1%)	
III	3	1 (33.3%)	2 (66.7%)	
Myometrial invasion				
< Half myometrial thickness	34	30 (88.2%)	4 (11.8%)	**0.05***
> Half myometrial thickness	26	17 (65.4%)	9 (34.6%)	
Cervical invasion				
Absent	38	32 (84.2%)	6 (15.8%)	0.20
Present	22	15 (68.2%)	7 (31.8%)	
Lymphovascular invasion				
Negative	43	40 (93%)	3 (7%)	**<0.001***
Positive	17	7 (41.2%)	10 (58.8%)	
MCM2 expression				
Low	28	26 (92.9%)	2 (7.1%)	**0.01***
High	32	21(65.6%)	11(34.4%)	
TGM2 expression				
Low	31	28 (90.3%)	3 (9.7%)	**0.03***
High	29	19 (65.5%)	10 (34.5%)	
TGM2 serum level				
Low	33	30 (90.9%)	3 (9.1%)	**< 0.01***
High	27	17 (63.0%)	10 (37.0%)	

In the present study, we investigated the immunohistochemical expression of TGM2, as well as its serum blood level in EC patients. High TGM2 immunohistochemical expression was found in 48.3% of our cases. There was a significant relation between TGM2 expression and high tumor grade, the presence of myometrial, cervical and lymphovascular invasion.

In an immunohistochemical study made by Jin et al., 2012 they reported that high TGM2 expression was associated with the presence of lymph nodal metastasis. The results of this study showed that high TGM2 could have an independent predictive factor for poor survival in those patients.

Wang et al., 2016 observed that TGM2 was highly expressed in gastric carcinoma, and that TGM2 promoted cellular proliferation, tumorigenesis, invasion, and peritoneal metastasis in vivo.

Regarding TGM2 serum levels in our study, high TGM2 serum levels were detected in 45% of cases. Our results showed that high TGM2 serum level showed a statistically significant relation with serous type EC, and myometrial invasion, which suggest that TGM2 blood level may related to worse prognosis in such patients. This was in contrast with the findings of Lan et al., 2020 who reported that TGM2 serum level was not significantly related to myometrial invasion.

Table 6. Univariate analysis of clinicopathological parameters and 3-years disease free survival (DFS) and relapse

Clinicopathological parameters		DFS (Months)	OR Relapse	95% CI for OR		P-value
				Lower	Upper	
Age (Years)	≤ 60 (n=26)	34.9 ± 8.9				
	> 60 (n=34)	34.4 ± 9.2	0.86	0.25	2.97	0.87
Histological type	Endometrioid (n=45)	35.6 ± 7.7				
	Serous (n=15)	31.7 ± 11.8	2.31	0.62	8.64	0.21
Histopathologic grade	Grade I (n=9)	34.9 ± 10.3				
	Grade II (n=27)	35.7 ± 8.5	1.82	0.18	18.04	0.61
	Grade III (n=24)	33.3 ± 9.2	3.29	0.35	31.49	0.30
FIGO stage	Stage I (n=25)	37.6 ± 5.4				
	Stage II (n=32)	33.7 ± 9.1	4.50	0.88	23.14	0.07
	Stage III (n=3)	20.3 ± 18.0	23.00	1.40	378.90	**0.03***
Myometrial invasion	< Half Thickness (n=34)	37.1 ± 6.0				
	> Half Thickness (n= 26)	31.3 ± 11.1	3.97	1.06	14.86	**0.04***
Cervical invasion	Absent (n=38)	35.9 ± 7.4				
	Present (n= 22)	32.4 ± 11.1	2.49	0.71	8.70	0.15
Lymphovascular invasion	Negative (n= 43)	37.6 ± 5.4				
	Positive (n= 17)	27.2 ± 11.8	19.05	4.17	87.06	**<0.001***
MCM2 expression	Low (n=28)	37.2 ± 6.3				
	High (n=32)	32.3 ± 10.4	6.81	1.36	34.16	**0.02***
TGM2 expression	Low (n=31)	36.6 ± 8.4				
	High (n=29)	32.5 ± 9.2	4.91	1.19	20.23	**0.03***
TGM2 serum level	Low (n=33)	38.3 ± 4.2				
	High (n=27)	30.1 ± 11.0	5.88	1.42	24.36	**0.02***

OR= Odds ratio, CI= Confidence interval

However, they estimated the prognostic benefits of serum TGM2 by measuring its pre- and post-operative level in EC patients. Serum TGM2 levels decreased to zero postoperatively, indicating it might be a potential follow-up marker for EC. In our study, no significant relation was observed between TGM2 serum level and age, histological grade, FIGO stage, cervical and lymphovascular invasion. Similar to our results, Torres et al., 2019, reported that TGM2 serum levels were not correlated with age or FIGO stage.

Regarding TGM2 serum levels, Eckert, 2019, Torres et al., 2019, and Lan et al., 2020 reported significantly higher serum TGM2 levels in EC compared to control and benign groups. Urick et al., 2021 stated also that TGM2 is a potential serum biomarker for EC. In the current study, the 3-years DFS was significantly associated with TGM2 immunohistochemical expression and TGM2 serum levels. Our results showed that high TGM2 serum level was statistically significant associated with shorter DFS, and higher incidence of relapse. Several factors could explain the association between high TGM2 expression and poor overall survival. Lei et al., 2018 reported that TGM2 endothelial expression in the newly formed blood vessels may promote tumor growth and metastasis.

TGM2 can activate nuclear factor-kappa (NF-κB); which therefore contributes to tumor formation. TGM2 can regulate the expression of genes which promote cancer cell survival and protect malignant cells from apoptosis. Kumar et al., 2012 observed that TGM2 could promote angiogenesis by activating HIF1α to subsequently induce vascular endothelial growth factor (VEGF) expression. TGM2 is reported to induce chemoresistance in many cancer types. Therefore, focusing on the role of TGM2 in cancer therapy is essential to develop new therapeutic targets.

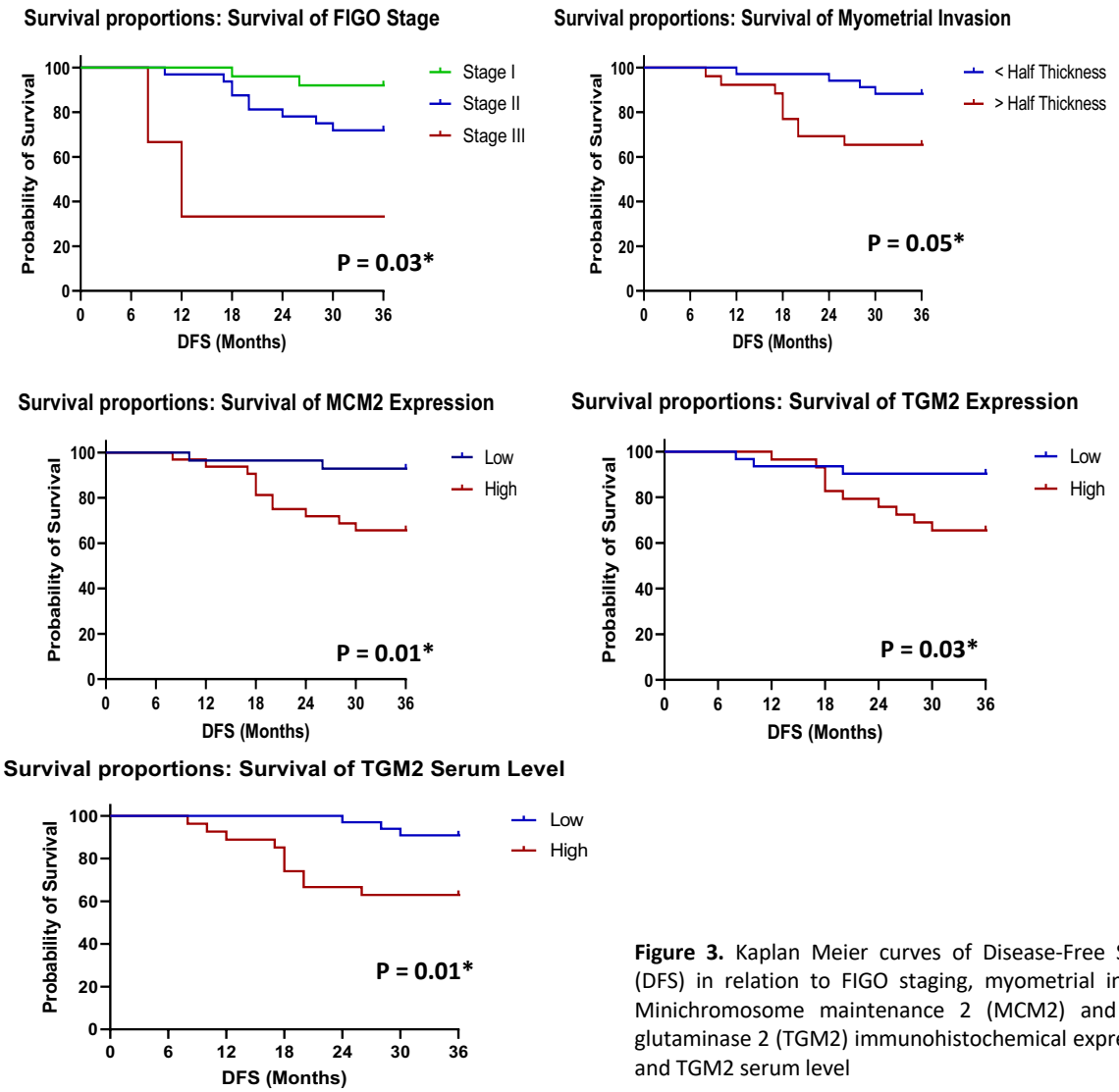

Figure 3. Kaplan Meier curves of Disease-Free Survival (DFS) in relation to FIGO staging, myometrial invasion, Minichromosome maintenance 2 (MCM2) and Trans-glutaminase 2 (TGM2) immunohistochemical expressions, and TGM2 serum level

He et al., 2015 reported that the inhibition of TGM2 using siRNA affected the expression of TGM2, vimentin, E-cadherin, and Bcl-2 and promoted apoptosis in drug-resistant cancer cells. Li et al., 2018 reported that the downregulation of TGM2 enhanced the chemosensitivity in cisplatin-chemoresistance osteosarcoma cells. Assi et al., 2013 also hypothesized TGM2 knockdown could reverse epithelial-mesenchymal transition (EMT), promote apoptosis and increase the chemosensitivity in cancer patients. Therefore, TGM2 may be an important prognostic factor for chemotherapy efficacy and could be used as effective therapeutic target for cancer patients.

CONCLUSIONS

Our study indicates that high MCM2 and TGM2 immunohistochemical expressions as well as high serum TGM2 levels are significantly associated with unfavorable prognosis and poor survival outcomes in EC patients. Further studies are required to evaluate their possible role as therapeutic targets in such patients.

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