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ORIGINAL ARTICLE

Efficacy of metformin in the treatment of estrogen-dependent endometrial carcinoma complicated with type 2 diabetes mellitus and analysis of its prognosis

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Summary

Purpose: *To explore the efficacy of metformin in the treatment of estrogen-dependent endometrial carcinoma (EC) complicated with type 2 diabetes mellitus (T2DM), and the influencing factors for the prognosis of such patients.*

Methods: *The clinical data of 68 patients histopathologically diagnosed with estrogen-dependent EC complicated with T2DM in our hospital from April 2013 to March 2016, and 132 estrogen-dependent EC patients with normal blood glucose during the same period were retrospectively analyzed. The clinical and pathological features were compared between diabetic patients and non-diabetic patients. The diabetic patients were divided into the metformin group and the non-metformin group according to whether metformin was taken. The survival curves were plotted and analyzed using the Kaplan-Meier method, and the overall survival (OS) and progression-free survival (PFS) were compared among the three groups. Moreover, the multivariate analysis was performed using the COX regression model, so as to analyze the influencing factors for the prognosis of patients with estrogen-dependent EC complicated with T2DM.*

Results: *Compared with non-diabetic patients, diabetic patients had higher age of onset, a higher BMI, higher proneness to hypertension, more advanced tumor stage, a higher histological grade, deeper myometrial invasion and a higher risk of lymph node metastasis. Both OS and PFS of T2DM patients who took metformin were significantly prolonged compared with those of T2DM patients who did not take metformin (p=0.021, p=0.011). There were no statistically significant differences in the PFS and OS between diabetic patients who took metformin and non-diabetic patients (p>0.05). According to the results of Cox multivariate analysis, OS was obviously shortened in case of high age of onset, complicated T2DM, late pathological stage of tumor advanced tumor stage, high histological grade, deep myometrial invasion and positive lymph node metastasis, while PFS could was obviously shortened in case of complicated T2DM, late pathological stage of tumor advanced tumor stage, high histological grade, deep myometrial invasion and positive lymph node metastasis. Metformin evidently improved OS and PFS.*

Conclusion: *Complicated T2DM, high age of onset, advanced tumor stage, high histological grade, deep myometrial invasion and positive lymph node metastasis are factors for the poor prognosis of patients with estrogen-dependent EC, and metformin can significantly ameliorate both OS and PFS in these patients, thereby improving their prognosis.*

Key words: *metformin, type 2 diabetes mellitus, estrogen-dependent endometrial carcinoma, prognosis*

Introduction

Endometrial carcinoma (EC), similarly with cervical and ovarian carcinoma, is one of the three major malignant tumors of the female reproductive system, accounting for about one fifth to one third of malignancies of the female genital tract. EC refers to a group of epithelial malignant tumors that originate in the endometrium, 75-90% of which are estrogen-dependent, namely type I EC. EC fre-

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quently occurs in perimenopausal women, and obesity, diabetes mellitus (DM) and complicated hypertension are high risk factors [1,2]. It has been confirmed in evidence-based medicine that patients with type 2 DM (T2DM) often have an increased risk of EC due to obesity and insulin resistance, and EC patients are mostly complicated with T2DM [3]. Metformin, as a first-line hypoglycemic agent for T2DM, is widely used in the treatment of various diseases accompanied by insulin resistance, such as polycystic ovary syndrome and metabolic syndrome, as well as for the prevention of DM. Recent studies have shown that metformin can reduce the morbidity and mortality rates of EC through various mechanisms, so it is expected to be used as a new type of anticancer drug in the prevention and treatment of EC [4,5].

In the present study, the clinical data of 68 estrogen-dependent EC patients complicated with T2DM treated in our hospital from April 2013 to March 2016, and 132 estrogen-dependent EC patients with normal blood glucose during the same period were retrospectively analyzed. The clinical and pathological features were compared between diabetic and non-diabetic patients, the efficacy of metformin on estrogen-dependent EC complicated with T2DM was explored, and the influencing factors for the prognosis of patients were analyzed, in the hope of providing basis for the treatment of such patients.

Methods

General data

The clinical data of 200 patients with estrogen-dependent EC treated in our hospital from April 2013 to March 2016 were retrospectively analyzed, including 68 estrogen-dependent EC patients complicated with T2DM (T2DM group) and 132 estrogen-dependent EC patients with normal blood glucose (Control group). In the T2DM group, 36 patients used to take or were taking metformin orally (including in combination with other hypoglycemic agents) (Metformin group), and 32 patients never took metformin without undergoing standardized hypoglycemic therapy or were taking other drugs to control blood glucose (Non-metformin group). The patients with estrogen-dependent EC underwent surgery performed by the same group of experienced senior gynaecologists in our hospital, and they were positively diagnosed by senior pathologists through histopathologic examination. EC was staged based on the 2009 staging criteria of the International Federation of Gynecology and Obstetrics (FIGO). T2DM was diagnosed under the assistance of endocrinologists in our hospital. Exclusion criteria: patients whose complete medical data could not be obtained, such as the pathology report, or with who had non-estrogen-dependent EC according to the pathology report, those who, or whose families, refused to accept the postoperative standardized therapeutic regimen developed by physicians according to the clinical practice guidelines for EC, young patients who needed to preserve their fertility or underwent progesterone therapy, those receiving unsatisfactory cytoreductive surgery (CRS), or those receiving no operative treatment due to severe medical complications. All patients enrolled adhered to the *Declaration of Helsinki*, and signed the informed consent. This study was approved by the Ethics Committee of Chengdu Second People's Hospital. Signed written informed consents were obtained from all participants before the study.

Treatment methods

According to the clinical practice guidelines for EC, the patients with estrogen-dependent EC underwent the operative treatment performed by the same group of experienced senior gynaecologists in our hospital (CRS for advanced EC patients, with satisfactory efficacy, namely no visible lesions or residual lesions <1 cm. Comprehensive staging surgery for early EC patients). After the operation, the patients were treated with standardized treatment or followed-up based on clinical practice guidelines according to the results of the pathological examination.

Observation indexes

In this study, the follow-up started from the initial definitive diagnosis (namely the date on the postoperative pathology report) until March 2019, and the content included whether the patients had recurrence or metastasis of EC, the time of recurrence or metastasis as well as related treatment, drug therapy and blood glucose control for T2DM patients, and whether the patients died, time and specific cause of death. New tumor lesions occurring after standardized radical therapy and complete remission, whose histopathologic type was consistent with that of the primary lesions indicated tumor recurrence. Diagnostic criteria for recurrence and metastasis of EC: After the standardized treatment was discontinued for more than 6 months, the recurrence or metastasis of EC could be confirmed in one of the following three cases during follow-up, and other new cancers were excluded: 1) pelvic-abdominal or distant space-occupying lesions measurable in the gynecological or imaging examination, 2) no space-occupying lesions measurable in the clinical or imaging examination, but the level of serum tumor marker CA-125 continuously rose, and 3) pleuroperitoneal fluid confirmed by the clinical or imaging examination, and cancer cells found by the cytological examination. There were two observation end points in this study, namely progression-free survival (PFS, the time from the date on the postoperative pathology report to the date of confirmed tumor recurrence and metastasis) and overall survival (OS, the time from the date on the postoperative pathology report to the date of confirmed death from any cause). If the patient died due to causes other than tumor recurrence, and tumor recurrence and metastasis could not be confirmed by clinical evidence before death, the end point of PFS was defined as the time of death. If tumor recurrence or death

could still not be confirmed clinically before the end of follow-up, the end points of PFS and OS were defined as the time of the last follow-up.

Statistics

Statistical Product and Service Solutions (SPSS) 22.0 software (IBM, Armonk, NY, USA) was used for statistical analysis. The measurement data were expressed as mean ± standard deviation (x±s), and t-test was performed for the intergroup comparison. The enumeration data were expressed as rate (%), and x^2 test was performed for the intergroup comparison. The survival curves were plotted and analyzed using the Kaplan-Meier method. The log-rank test was used to detect whether PFS and OS had statistically significant differences among the three groups, while the correlations of each factor with PFS and OS were analyzed using Cox multivariate analysis. $p<0.05$ suggested statistically significant differences.

Results

Comparisons of clinical and pathological features

A total of 68 estrogen-dependent EC patients complicated with T2DM and 132 estrogen-dependent EC patients with normal blood glucose were enrolled in this study. The analysis of clinical data in both groups revealed that compared with the Control group, the T2DM group had higher age of onset [(58.35±8.73) years old *vs.* (55.67±7.80) years old, p=0.028], a higher BMI [(33.83±5.64) kg/m^2 *vs.* (26.33±5.95) kg/m^2, p=0.001], a more advanced tumor stage (a larger proportion of patients in stage III-IV, p=0.006), a higher histological grade, namely poorer tumor differentiation (p=0.026), deeper myometrial invasion (p=0.012), more frequent lymph node metastasis (p=0.043) and higher proneness to hypertension (p=0.040) (Table 1).

Comparison of patient survival

To analyze the effect of metformin treatment on the survival time of estrogen-dependent EC patients complicated with T2DM, the clinical baseline data were first compared between the Metformin group and the Non-metformin group. The results showed that there were no statistically significant differences in the age of onset, BMI, tumor stage, histological type, depth of myometrial invasion, presence or absence of lymph node metastasis and hypertension between the two groups (p>0.05), with comparable results (Table 2).

According to the follow-up results, at the end of the follow-up, the median OS and PFS were 48.6±5.0 and 48.3±5.4 months in the Metformin group, 36.9±5.1 and 34.4±4.8 months in the Non-metformin group, and 46.6±5.3 and 43.5±5.0 months in the Control group. Besides, the survival curves of patient's OS and PFS were plotted using the Kaplan-Meier method (Figure 1). The results of the log-rank test revealed that OS in the Non-metformin group was significantly shorter than that in the Metformin group (p=0.021) and the Control group (p=0.044), while PFS in the Non-metformin group was also significantly shorter than that in the Metformin group (p=0.011) and the Control group (p=0.007), showing statistically significant

Table 1. Comparison of clinical and pathological characteristics of patients in T2DM group and Control group

Parameters	*T2DM group n=68*	*Control group n=68*	*p value*
Age, years	58.35±8.73	55.67±7.80	0.028
BMI (kg/m^2)	33.83±5.64	26.33±5.95	0.001
FIGO stage, n (%)			0.006
I	17 (25.0)	67 (50.8)	
II	29 (42.6)	35 (26.5)	
III	18 (26.5)	25 (18.9)	
IV	4 (5.9)	5 (3.8)	
Histologic grade, n (%)			0.026
G1	23 (33.8)	71 (53.8)	
G2	28 (41.2)	40 (30.3)	
G3	17 (25.0)	21 (15.9)	
Depth of myometrial invasion, n (%)			0.012
No or shallow	38 (55.9)	97 (73.5)	
Deep	30 (44.1)	35 (26.5)	
Lymph node metastasis	11 (16.2)	10 (7.6)	0.043
Hypertension	31 (45.6)	41 (31.1)	0.040

T2DM: Type 2 diabetes mellitus; BMI: Body Mass Index; FIGO: International Federation of Gynecology and Obstetrics

differences. Both OS and PFS in the Metformin group were longer than those in the Control group, but the differences were not statistically significant (OS: p=0.586, PFS: p=0.897).

Cox multivariate analysis of prognosis

The clinical and pathological data were sorted out, and 7 variables were determined: age of onset, BMI, whether T2DM was complicated, whether metformin was taken, tumor stage, histological grade, depth of myometrial invasion, and lymph node metastasis, followed by Cox multivariate analysis using the SPSS 22.0 software, so as to explore the influencing factors for the prognosis of estrogen-dependent EC (Tables 3 & 4). It was found that the age of onset, whether T2DM was complicated, whether metformin was taken, tumor stage, histological grade, depth of myometrial invasion, and lymph node metastasis were influencing factors for the OS of estrogen-dependent EC patients. OS was shortened under in case of high age of onset, complicated T2DM, advanced tumor stage, high histological grade, deep myometrial invasion and positive lymph node metastasis, while metformin evidently improved OS. Moreover, whether T2DM was complicated, whether metformin was taken, tumor stage, histological grade, depth of myometrial invasion, and lymph node metastasis were influencing factors for the PFS of estrogen-dependent EC patients, PFS was obviously shortened under in

Table 2. Baseline clinical and pathological characteristics of estrogen-dependent endometrial carcinoma patients with T2DM

Parameters	*Metformin group n=36*	*Non-Metformin group n=32*	*p value*
Age, years	59.07±8.83	58.31±9.24	0.730
BMI (kg/m^2)	32.82±4.48	34.37±3.87	0.134
FIGO stage, n (%)			0.444
I-II	26 (72.2)	20 (62.5)	
III-IV	10 (27.8)	12 (37.5)	
Histologic grade, n (%)			0.836
G1	13 (33.8)	10 (53.8)	
G2	15 (41.2)	13 (30.3)	
G3	8 (25.0)	9 (15.9)	
Depth of myometrial invasion, n (%)			0.666
No or shallow	21 (55.9)	17 (73.5)	
Deep	15 (44.1)	15 (26.5)	
Lymph node metastasis, n (%)	5 (16.2)	7 (7.6)	0.389
Hypertension, n (%)	14 (45.6)	17 (31.1)	0.239

T2DM: Type 2 diabetes mellitus; BMI: Body Mass Index; FIGO: International Federation of Gynecology and Obstetrics

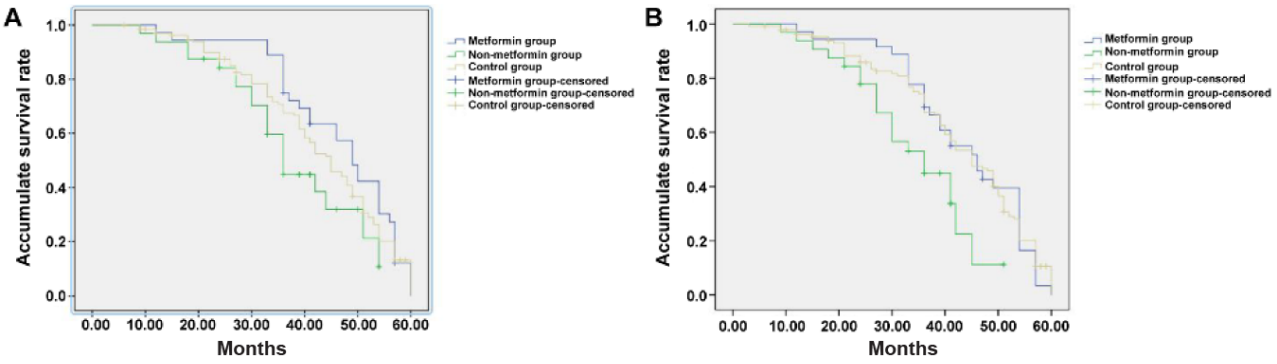

Figure 1. Kaplan-Meier survival curves of the studied patients. **A:** The overall survival rate of patients in the Metformin group was significantly higher than that in the Non-Metformin group (p=0.021). The difference between the overall survival rate of patients in the Metformin group and control group had no statistical significance (p=0.586). **B:** The progression-free survival rate of patients in the Metformin group was significantly higher than that in the Non-Metformin group (p=0.011). The difference between the progression-free survival rate of patients in the Metformin group and control group had no statistical significance (p=0.897).

Table 3. Multivariate analysis of predictors for overall survival time in patients with estrogen-dependent endometrial carcinoma

Parameters	*OR*	*95% CI*	*p value*
Age	1.23	1.08-1.57	0.034
BMI	2.63	0.93-6.16	0.112
T2DM	1.54	1.38-3.36	0.008
Metformin	0.46	0.30-0.93	0.011
FIGO stage	2.24	1.18-7.71	0.006
Histologic grade	1.39	1.14-1.95	0.019
Muscular invasion	1.17	1.04-1.68	0.045
Lymph node metastasis	1.89	1.57-8.73	0.010

OR: Odds ratio; CI: Confidence interval; T2DM: Type 2 diabetes mellitus; BMI: Body Mass Index; FIGO: International Federation of Gynecology and Obstetrics

Table 4. Multivariate analysis of predictors for progression-free survival time in patients with estrogen-dependent endometrial carcinoma

Parameters	*OR*	*95% CI*	*p value*
Age	2.09	0.95-3.24	0.074
BMI	2.35	0.86-5.40	0.093
T2DM	1.83	1.21-4.69	0.007
Metformin	0.41	0.21-0.87	0.009
FIGO stage	2.79	1.32-8.91	0.001
Histologic grade	1.26	1.16-1.82	0.027
Muscular invasion	1.33	1.08-1.38	0.031
Lymph node metastasis	2.89	1.34-9.31	0.003

OR: Odds ratio; CI: Confidence interval; T2DM: Type 2 diabetes mellitus; BMI: Body Mass Index; FIGO: International Federation of Gynecology and Obstetrics

case of complicated T2DM, advanced tumor stage, high histological grade, deep myometrial invasion and positive lymph node metastasis, while metformin evidently improved PFS.

Discussion

EC is one of the three major malignant tumors of the female reproductive system, and its morbidity rate shows an increasing trend in recent years due to changes in lifestyle. DM is one of the high-risk factors for EC. Metformin can improve insulin resistance and serve as a classical therapeutic drug for DM. According to recent studies, metformin possesses an anticancer effect, and it is expected to play an important role in the prevention and treatment of EC since it has an effect on the high-risk factors for EC and the tumor itself.

In recent years, studies have demonstrated that metformin can reduce cancer risk and improve the survival rate of T2DM patients with breast, prostate, colorectal and head-neck cancer [4,6,7]. It has been confirmed through *in vitro* experiments that metformin can inhibit the proliferation and growth of a variety of human malignant tumor cells, including gastric cancer, pancreatic cancer, medullary thyroid carcinoma and EC, in a dose-dependent manner, cause cell cycle arrest and apoptosis, and reduce invasion and metastasis [8-10]. Related research has revealed that metformin is able to enhance the sensitivity to radiotherapy, and combined radiotherapy can effectively inhibit the proliferation and local recurrence of tumor cells, and improve prognosis [11]. The retrospective studies and basic experimental studies by Kitazon and Rocha et al showed that the synergistic anticancer effect of metformin with chemotherapeutic drugs can effectively enhance the chemotherapeutic efficacy, and that lower-dose chemotherapeutic drugs can achieve therapeutic effects, reduce the incidence of adverse chemotherapy reactions, lower the resistance to chemotherapeutic drugs, selectively kill cancer stem cells, and reduce the risk of tumor recurrence [12]. Progesterone therapy, one of the effective treatment measures for EC, is mainly used in patients with advanced inoperable cancer or recurrent cancer, as well as in young patients with early EC in order to preserve fertility. Met-

formin can directly activate the expression of the progesterone receptor through activating AMPK, and also activate its transcription through inhibiting IGF-1/P13K/Akt, thereby reversing progesterone resistance. The newly-discovered cross-talk between insulin and estrogen signaling pathways may also play a role [13]. It is reported in multiple retrospective cohort studies that the survival rate of T2DM patients treated with metformin is significantly improved and the risk of EC-related death is greatly reduced compared with that of T2DM patients not treated with metformin and patients without T2DM [14,15]. The anti-EC effect of metformin may be related to the ATM-LKB1-AMPK-mTOR signaling pathway and its inhibition of cell cycle, as well as to the regulation of insulin and insulin-like growth factor levels and the selective killing of cancer stem cells [16-19].

Studies have shown that the level of serum insulin in patients with EC is significantly higher than that in normal people, and insulin resistance is more common in patients with EC than in normal healthy people [20]. It can be reasonably speculated based on the above association that insulin resistance is related to the occurrence and development of EC, and such metabolic syndromes as DM, hypertension and obesity [21]. In this study, it was found that estrogen-dependent EC patients complicated with T2DM had higher age of onset, a higher BMI, a more advanced tumor stage, a higher histological grade, namely poorer tumor differentiation, deeper myometrial invasion, more frequent lymph node metastasis, and higher proneness to hypertension. According to follow-up results, metformin significantly prolonged both the PFS and OS of estrogen-dependent EC patients complicated with T2DM. The results of Cox multivariate analysis showed that high age of onset, advanced tumor stage, complicated T2DM, high histological grade, deep myometrial invasion and lymph node metastasis were factors for the poor prognosis of these patients, consistent with the literature reports.

The present study has several limitations. For example, the sample size was small, the follow-up time was insufficient, the influence of medical complications, administration dose and time of metformin, and different therapeutic regimens were not analyzed, some patients failed to be regularly reexamined, and there was deviation in the accurate time of tumor recurrence and metastasis, all of which affected the prognostic analysis of EC patients. In the future, further large-sample multicenter randomized controlled trials are needed to verify the influencing factors for the prognosis of estrogen-dependent EC complicated with T2DM, as well as the association between metformin and DM prognosis and estrogen-dependent EC and its mechanism.

Conclusions

Complicated T2DM, high age of onset, advanced tumor stage, high histological grade, deep myometrial invasion and positive lymph node metastasis are factors for the poor prognosis of patients with estrogen-dependent EC, and metformin can significantly ameliorate both the OS and PFS of these patients, thereby improving their prognosis.

Conflict of interests

The authors declare no conflict of interests.

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