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CLINICAL TRIAL REPORT

Metformin plus first-line chemotherapy versus chemotherapy alone in the treatment of epithelial ovarian cancer: a prospective open-label pilot trial

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Abstract

Purpose To evaluate the efficacy of metformin plus first-line chemotherapy versus chemotherapy alone in the treatment of epithelial ovarian cancer.

Methods Epithelial ovarian cancer patients without diabetes mellitus were allocated to non-metformin group (paclitaxel plus carboplatin) or metformin group (paclitaxel plus carboplatin plus metformin). The primary endpoint was progression-free survival (PFS) and disease-free survival (DFS).

Results A total of 20 patients were assigned to metformin group and 24 patients to non-metformin group. The baseline information in two groups had no significant difference. The PFS and DFS of patients with metformin intake versus without metformin intake was 23 versus 21 months ($p=0.68$) and 29 versus 26 months ($p=0.61$), respectively. The PFS and DFS of patients with normal weight versus obese/overweight were 23 versus 17 months ($p=0.14$) and 27 versus 23 months ($p=0.50$), respectively. Metformin effectively inhibited the increase of IGF-1 and maintained the IGFBP-1.

Conclusions Within the limitations of the small sample size, there was no evidence of meaningful effect on PFS by metformin even though evidence of modulation of IGF-1 signaling axis was apparent.

Keywords Epithelial ovarian cancer · Clinical trial · Metformin · First-line chemotherapy

Introduction

Increasing epidemiological studies and meta-analysis have indicated that hyperinsulinemia and insulin resistance status play key roles in the initiation and progression of various human malignant tumors [1, 2]. This point was consistent with the finding in a variety of large population-based cohort studies that patients with diabetes and neoplasms had poorer prognosis than those without diabetes including ovarian cancer [3–7]. In addition, elevated body mass index (BMI) was associated with increased risk of ovarian cancer [8]. Biologically speaking, insulin could stimulate DNA synthesis and regulate cell differentiation and promote cell proliferation by cross-activating insulin-like growth factor (IGF) receptors [9]. Other studies confirmed that IGF/insulin system promoted malignancies development including breast cancer and ovarian cancer by stimulating mitogenesis and migration [10–12]. Activating the tyrosine kinase growth receptor pathway has been elucidated as the downstream pathway of the IGF/insulin system [13]. Once the receptors were activated, the insulin receptor-substrate-2 will be upregulated, which will lead to the activation of MAPKinase and PI3K-Akt signaling pathway [14]. The bioactivity of IGFs could be regulated by IGF binding proteins (IGFBPs) which bind to IGFs with high affinity and attenuate IGF access to IGF1R [15]. IGFBPs family consists of 7 members (IGFBP-1-7) [11] and several studies showed that IGFBPs were associated with poor prognosis of malignancies [16, 17]. Based on the evidence above, drugs targeting the signaling pathway could be explored.

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Metformin, widely used in the treatment of type II diabetes mellitus for decades, has been found being beneficial to prognosis of cancer patients [18, 19]. Generally, metformin exerts its anti-neoplastic activity both in an indirect and a direct way. Indirectly, metformin inhibits gluconeogenesis and glucose output from liver which decreases glucose level in serum systematically followed by decreased insulin level [20]. Directly, metformin activates the LKB1–AMPK pathway and inhibits the downstream mTOR, which leads to inhibition of p70S6Ks and 4E-BP-1 phosphorylation and protein synthesis in cancer [21, 22]. Our preclinical studies found that metformin could attenuate the proliferation and metastasis of ovarian cancer in vitro and in vivo [23, 24]. Another clinical study on endometrial atypical hyperplasia found that the complete response rate in metformin plus megestrol acetate group was higher than that in megestrol acetate group (75% versus 25%) [25].

In recent years, prospective clinical trials on metformin's anti-cancer effects on breast cancer, non-small cell lung cancer, prostate cancer and pancreatic cancer have been published [26–29]. In human epidermal growth factor receptor 2 (HER2)-positive breast cancer, patients with metformin addition had a better survival than those with chemotherapy alone [30]. Till now, little prospective clinical data about the effect of metformin on prognosis of ovarian cancer patients have been found. The present study aimed to evaluate the anticancer effect of metformin plus first-line chemotherapy in epithelial ovarian cancer.

Materials and methods

Study design and subjects

This clinical trial was a prospective, open-label, randomized pilot study performed between June 2016 and March 2019 in the Obstetrics and Gynecology Hospital of Fudan University aimed at evaluating the efficacy of addition of metformin to first-line chemotherapy on epithelial ovarian cancer. The study was approved by the Ethics Committee of the Obstetrics and Gynecology Hospital of Fudan University and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants. The clinical trial was registered in Chinese Clinical Trial Registry (ChiCTR-IOR-17011859).

The inclusion criteria were as follows: (1) histologically diagnosed epithelial ovarian cancer including serous cancer, mucinous cancer, endometrial cancer and clear cell cancer; (2) age 18–75 years; (3) normal liver and kidney function (alanine transaminase <66 U/L, aspartate transaminase <36 U/L, total bilirubin <22 μmol/L, crea <106 μmol/L, blood urea nitrogen <6.1 mmol/L); (4) normal bone marrow function (neutrophils $\geq 1800/mm^3$ and platelets $\geq 80{,}000/mm^3$). The exclusion criteria were as follows: (1) radiotherapy or chemotherapy history; (2) other malignant tumor history; (3) pregnancy; (4) mental disorder; (5) diabetes mellitus history (at least two of three glycemic criteria: fasting plasma glucose level $\geq$ 7.0 mmol/L or random glucose $\geq$ 11.1 mmol/L or glycated hemoglobin level $\geq 6.5\%$); (6) contraindication of metformin.

Procedures and treatment

All patients underwent general physical examination, blood tests, pelvic computed tomography (CT) or magnetic resonance imaging (MRI) and chest X-ray before operation. All patients receive primary debulking surgery. Eligible patients were randomly allocated to non-metformin group (paclitaxel 135 mg/m^2 intravenously plus carboplatin area under the curve 5 intravenously or intraperitoneally) or metformin group (paclitaxel 135 mg/m^2 intravenously plus carboplatin area under the curve 5 intravenously or intraperitoneally plus metformin 850 mg/day). Chemotherapy treatment was administered every 3 weeks for 6–8 cycles. Metformin was administered orally every night post-dinner from the first day to the end day of chemotherapy. Baseline serum (fasting serum on the day before the first chemotherapy) and post-intervention serum (fasting serum on the day after the last chemotherapy) of all eligible patients were collected and stored at -80 °C. Fresh cancer tissues of all eligible patients were collected during operation and fixed in paraformaldehyde. All eligible patients underwent physical examination, blood test for CA125 and HE4, pelvic ultrasonography, electrocardiography and chest X-ray at least every three cycles. Disease status of all patients was evaluated by experienced gynecological doctors every two cycles. Once treatment was completed, patients were ordered to visit doctors every 3 months within 2 years and every 6 months 2 years later. Blood test for CA125 and HE4, pelvic ultrasonography and CT/MRI if necessary were performed to visited patients aiming to assess disease status.

Enzyme-linked immunosorbent assay and Luminex assay

IGF-1, insulin C-peptide, IGFBP-1 and IGFBP-7 levels in serum at baseline and post-intervention were tested according to instructions of manufacturer with Luminex Human Magnetic Assay (R&D Systems, Bio-Techne Corporation, MN, USA) and Human IGF-1 Quantikine ELISA Kit (R&D Systems, Bio-Techne Corporation, MN, USA).

Immunohistochemistry

Fresh tumor tissues of all eligible patients were fixed in paraformaldehyde and embedded in paraffin. The embedded

tissues were sliced into 5-μm-thick sections and deparaffinized, dehydrated and underwent antigen retrieval at 95 °C for 30 min. After blocked with goat serum, slides were incubated with primary antibodies overnight and HRP-conjugated secondary antibodies for 45 min. DAB (Jiehao Biotechnology, China) was used for color development. The slides were examined under the microscope, and the representative images were captured. For each slide, five visual fields were randomly selected. The anti-Ki-67, anti-insulin receptor, anti-*p*-mTOR and anti-*p*-4EBP-1 antibodies were purchased from Cell Signaling Technology (USA). Image-Pro Plus 6.0 (Media Cybernetics, USA) was used to analyze the captured images.

Outcomes

The primary endpoint of this study was progression-free survival (PFS) calculated from the last day of chemotherapy to the date of disease progression, death from any cause or loss to follow-up and disease-free survival (DFS) calculated from the day of debulking surgery to the date of disease progression, death from any cause or loss to follow-up. PFS or DFS of patients with no disease progression who were lost to follow-up was considered as censored values. Disease progression was evaluated with the Response Evaluation Criteria in S olid Tumors (RECIST version 1.1). The second endpoint was the improvement of serum biomarkers after interventions.

Statistical analysis

SPSS 16.0 (IBM Co., Armonk, NY, USA) was used to perform statistical analysis. Chi-square test was performed to analyze count data. Independent *t* test was used to analyze quantitative data in two groups. Paired *t*-test was used to compare biomarker changes after interventions. PFS and DFS were described using the Kaplan–Meier curves and compared with the log-rank test. A $p < 0.05$ was considered as significant statistically.

Results

Participants' characteristics

A total of 49 participants were enrolled in this study initially. Among them, two patients dropped out and two patients received other chemotherapy regimens because of platinum insensitivity and one lost to follow-up. The study profile is represented in Fig. 1. Finally, 20 patients were assigned to metformin group and 24 patients to non-metformin group. The general characteristics are shown in Table 1. The mean age of patients in metformin group was 53.55 ± 9.20 years

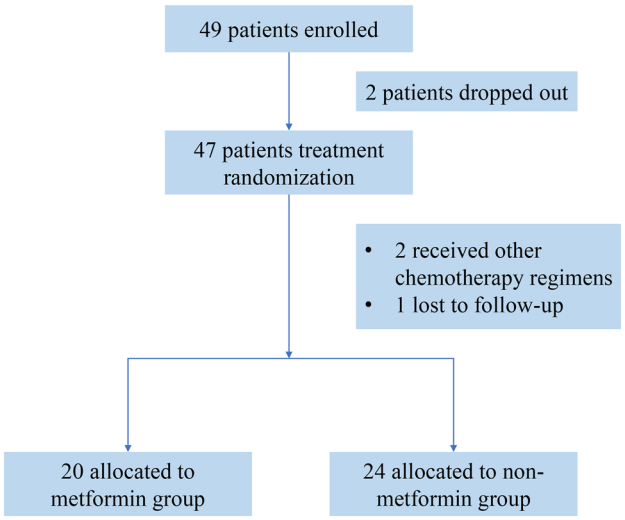

Fig. 1 Study profile

(mean ± standard deviation, SD) and 52.88 ± 8.77 years in non-metformin group. The general information in two groups had no significant difference, including body mass index (BMI) ($p = 0.725$), FIGO stage ($p = 0.908$), Grade, lymph node metastasis ($p = 0.138$) and so on.

Ki-67, insulin receptor, *p*-mTOR and *p*-4EBP-1 were upregulated in advanced ovarian cancer tissue

Ovarian cancer tissues were collected from all eligible patients. Results from our study presented that the expression of Ki-67, insulin receptor, *p*-mTOR and *p*-4EBP-1 was obviously upregulated in advanced ovarian cancer compared with that in early-stage patients (Fig. 2). Data from TCGA database showed that Ki-67, *p*-mTOR and *p*-4EBP-1 were overexpressed in ovarian cystadenocarcinoma compared with normal ovary tissue ($p < 0.05$); while insulin receptor did not (Fig. 3a–d). To analyze the association of expression levels of these biomarkers with PFS, we performed log-rank test and found that the PFS of patients with high Ki-67 expression was shorter than that with low expression ($p = 0.01$); while, no significant difference was found in insulin receptor, *p*-mTOR and *p*-4EBP-1 (Fig. 3e–h).

The effects of metformin or BMI on PFS and DFS

To compare the difference of PFS and DFS between metformin group and non-metformin group, Kaplan–Meier curves were made and log-rank test showed that the PFS and DFS of patients with metformin intake versus without metformin intake were 23 versus 21 months ($p = 0.68$) and 29 versus 26 months ($p = 0.61$), respectively (Fig. 4a, c). To study whether BMI will influence PFS or DFS of ovarian cancer patients, BMI of all participants was calculated.

Table 1 Patients demographics and baseline clinical characteristics

Characteristics	Metformin ($N=20$)	Non-metformin ($N=24$)	p
Age (years), mean ± SD	53.55 ± 9.20	52.88 ± 8.77	0.805
BMI (kg/m^2), mean ± SD	23.23 ± 3.75	23.60 ± 3.16	0.725
Preoperative CA125 (U/mL), mean ± SD	1364.50 ± 2340.05	1042.2 ± 1862.74	0.618
Preoperative HE4 (pmol/L), mean ± SD	375.73 ± 387.91	297.83 ± 348.82	0.519
FIGO stage			0.908
I + II	7 (35%)	8 (33.33%)	
III + IV	13 (65%)	16 (66.67%)	
Grade[a]			
Low	0	0	
High	15 (100%)	20 (100%)	
Postoperative residual disease (cm)			0.067
≤ 1	16 (80%)	20 (83.33%)	
> 1	4 (20%)	4 (16.67%)	
Chemotherapy			
Platinum	20 (100%)	24 (100%)	
Non-platinum	0	0	
None	0	0	
Histology			0.759
Serous	15 (75%)	20 (83.33%)	
Non-serous	5 (25%)	4 (16.67%)	
Lymph node metastasis[b]			0.138
Yes	3 (17.65%)	8 (40%)	
No	14 (82.35%)	12 (60%)	
Ascites			0.824
< 100 mL	11 (55%)	14 (58.33%)	
≥ 100 mL	9 (45%)	10 (41.67%)	

SD standard deviation, *BMI* body mass index, *FIGO* International Federation of Gynecology and Obstetrics

[a]Five patients in Metformin group and four patients in non-metformin group were clear cell carcinoma

[b]Three patients in Metformin group and four patients in non-metformin group did not receive lymph node dissection

Data showed that PFS and DFS of patients with normal weight versus obese/overweight were 23 versus 17 months ($p=0.14$) and 27 versus 23 months ($p=0.50$), respectively (Fig. 4b, d).

Serum biomarkers improvement after interventions

Fasting serum at baseline and post-treatment of all participants was collected. With Luminex assay and ELISA assay, the levels of IGF-1, insulin C-peptide, IGFBP-1 and IGFBP-7 were detected and analyzed. We found that the level of IGF-1 obviously increased at the end of treatment in the non-metformin group; while the increment was inhibited by adding metformin to the first-line chemotherapy in the metformin group ($p<0.001$) (Fig. 5a). Interestingly, results showed that IGFBP-1 which could bind to and decrease the serum level of IGF-1 declined significantly in the non-metformin group; while, the level of IGFBP-1 was maintained in the metformin group ($p<0.05$) (Fig. 5c). No significant changes were found in insulin C-peptide and IGFBP-7 after interventions (Fig. 5b, d). Additionally, we collected the level of CA125 and HE4 before and after interventions from medical history. The value of CA125 decreased by half 30 days after chemotherapy and to normal range (< 35 U/mL) 60 days after chemotherapy, which was considered as satisfaction. The value of HE4 decreased by half 30 days after chemotherapy and to normal range (< 40 years old: < 60.5 pmol/L; 40–49 years old: < 76.2 pmol/L; 50–59 years old: < 74.3 pmol/L; 60–69 years old: < 82.9 pmol/L; ≥ 70 years old: < 104 pmol/L) 60 days after chemotherapy, which was considered as satisfaction. The satisfaction rate of CA125 decline in the metformin group and the non-metformin group was 70.59% and 68.18%, respectively ($p=0.872$) (Table 2). The satisfaction rate of HE4 decline in the metformin group and the non-metformin group was 62.5% and 80%, respectively ($p=0.285$) (Table 3).

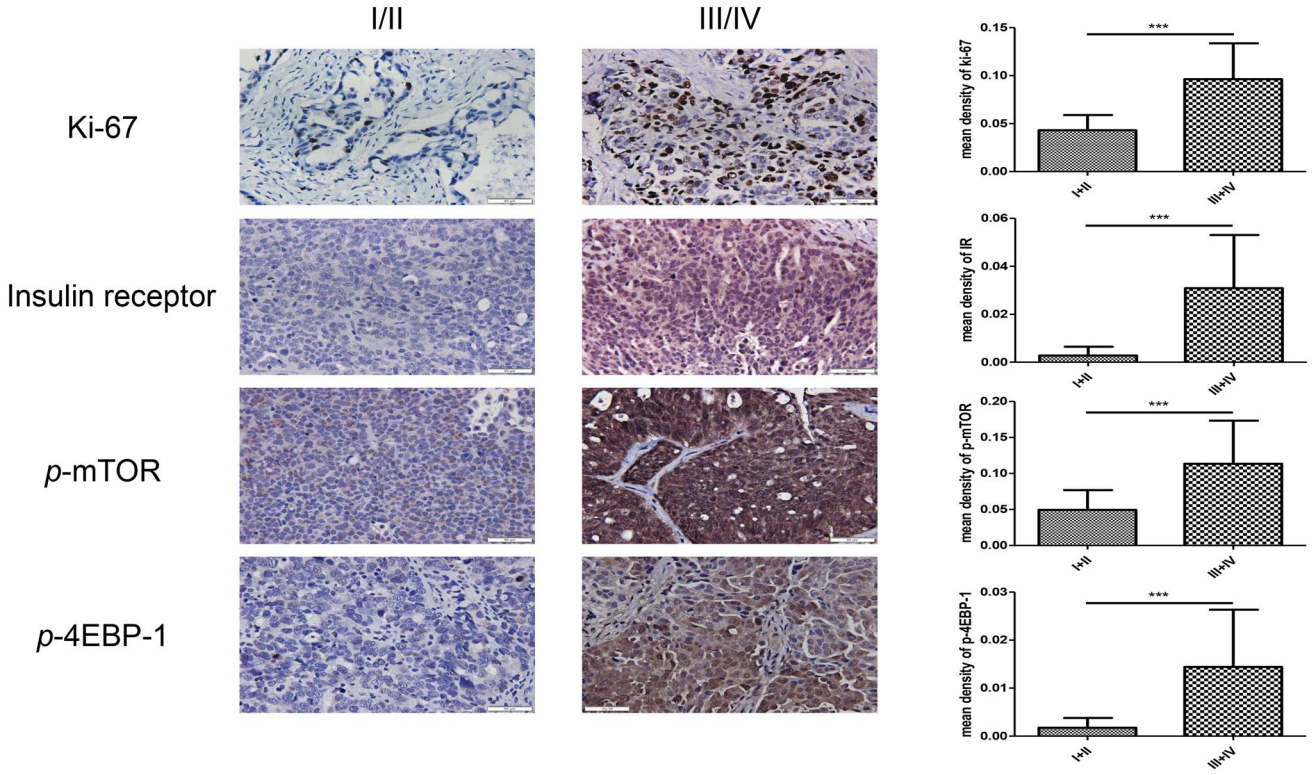

Fig. 2 The relative expression of Ki-67, Insulin receptor, *p*-mTOR and *p*-4EBP-1 in both early- and late-stage ovarian cancer tissues. Pre-treatment ovarian cancer tissues were collected from patients. Immunohistochemistry assay was performed to detect the expression of Ki-67, Insulin receptor, *p*-mTOR and *p*-4EBP-1. ***$p < 0.001$

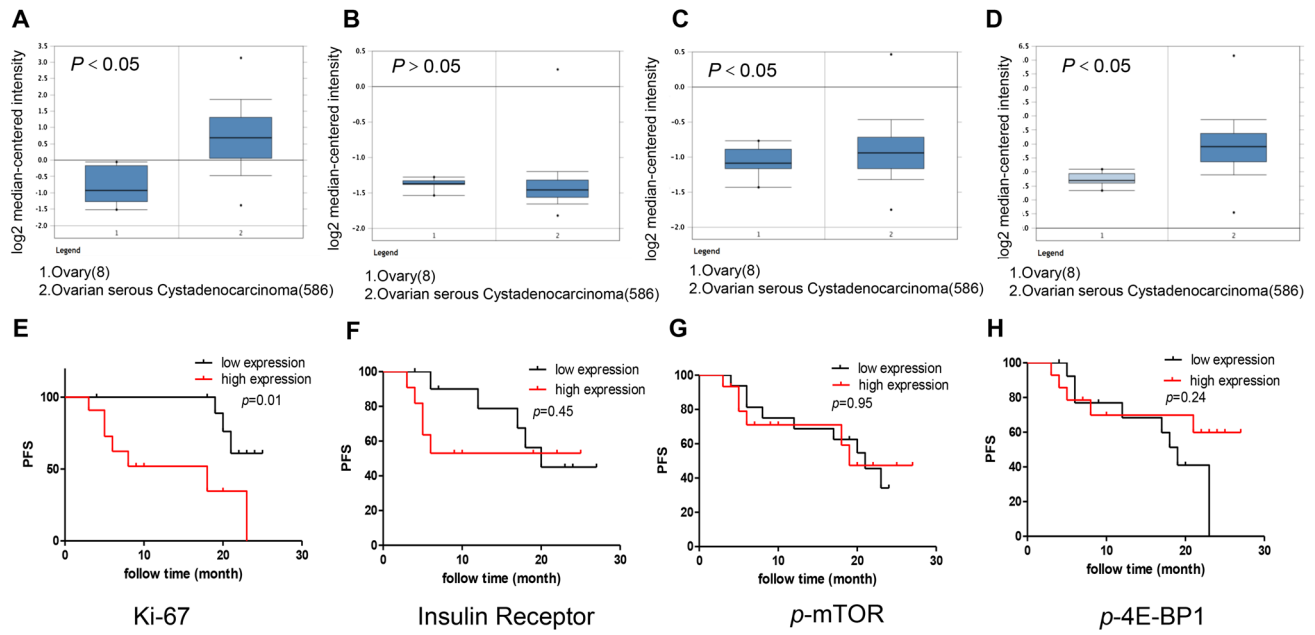

Fig. 3 The expression of Ki-67, Insulin receptor, *p*-mTOR and *p*-4EBP-1 in normal ovary and ovarian serous cystadenocarcinoma and the relationship between expression level and progression-free survival (PFS). **a**–**d** The relative expression of Ki-67, insulin receptor, *p*-mTOR and *p*-4EBP-1 in normal ovary and ovarian serous cystadenocarcinoma from TCGA database. **e**–**h** The PFS of recruited patients was followed up and Kaplan–Meier assay was used to analyze PFS of patients with low or high expression level of Ki-67, Insulin receptor, *p*-mTOR and *p*-4EBP-1 based on immunohistochemistry

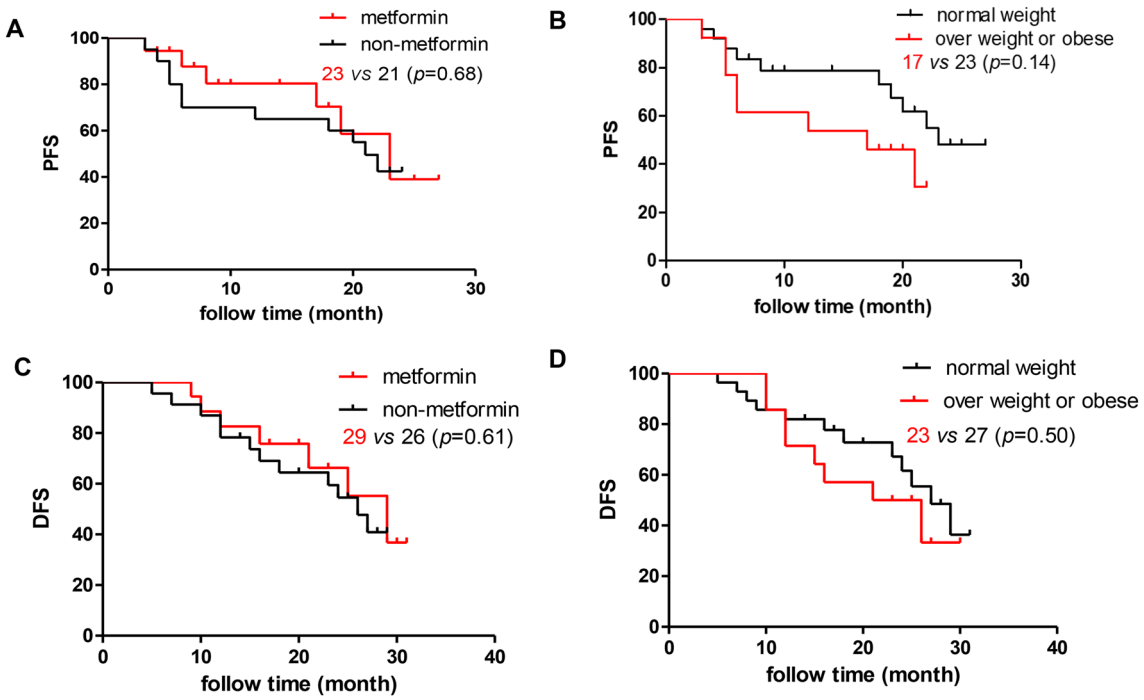

Fig. 4 The relationship between PFS or DFS and metformin intake or BMI. Kaplan–Meier assay was used to compare the PFS (**a**) and DFS (**c**) between patients with metformin intake or not. The PFS and DFS of patients with metformin intake versus without metformin intake was 23 versus 21 months ($p=0.68$) and 29 versus 26 months ($p=0.61$), respectively. **b**, **d** Kaplan–Meier assay was used to compare the PFS and DFS between ovarian cancer patients with normal weight (BMI < 24) and obese/overweight (BMI ≥ 24). The PFS and DFS of patients with normal weight versus obese/overweight was 23 versus 17 months ($p=0.14$) and 27 versus 23 months ($p=0.50$), respectively

Table 2 The decrease of CA125 after chemotherapy

	Metformin ($N=17$)[a]	Non-metformin ($N=24$)[b]	p
Satisfied[c]	12 (70.59%)	15 (68.18%)	
Non-satisfied	5 (29.41%)	7 (31.82%)	0.872

[a]The CA125 value of three patients in metformin group could not be found

[b]The CA125 value of two patient in non-metformin group could not be found

[c]The value of CA125 decreases by half 30 days after chemotherapy and to normal range (< 35 U/mL) 60 days after chemotherapy was considered as satisfaction

Table 3 The decrease of HE4 after chemotherapy

	Metformin ($N=16$)[a]	Non-metformin ($N=20$)[b]	p
Satisfied[c]	10 (62.5%)	16 (80%)	
Non-satisfied	6 (37.5%)	4 (20%)	0.285

[a]The HE4 value of four patients in metformin group could not be found

[b]The HE4 value of four patients in non-metformin group could not be found

[c]The value of HE4 decreases by half 30 days after chemotherapy and to normal range (< 40 years old: < 60.5 pmol/L; 40–49 years old: < 76.2 pmol/L; 50–59 years old: < 74.3 pmol/L; 60–69 years old: < 82.9 pmol/L; ≥ 70 years old: < 104 pmol/L) 60 days after chemotherapy was considered as satisfaction

Discussion

In the current study, the baseline characteristics of participants in two groups had no significant difference and no one quit the trial due to adverse effects. Results from the study showed that Ki-67, insulin receptor, *p*-mTOR and *p*-4EBP-1 were highly expressed in advanced stage ovarian cancer and patients with high Ki-67 and insulin receptor expression had a poorer PFS. As for the efficacy of metformin on prognosis of epithelial ovarian cancer, the PFS and DFS of patients in metformin group were 2 months and 3 months longer than that in non-metformin group, respectively. Although the p values were above 0.05, the improvement trend was noticeable. In addition, we compared the decrease of CA125 and HE4 which were the most common tumor makers in evaluating epithelial ovarian cancer after interventions and found no obvious

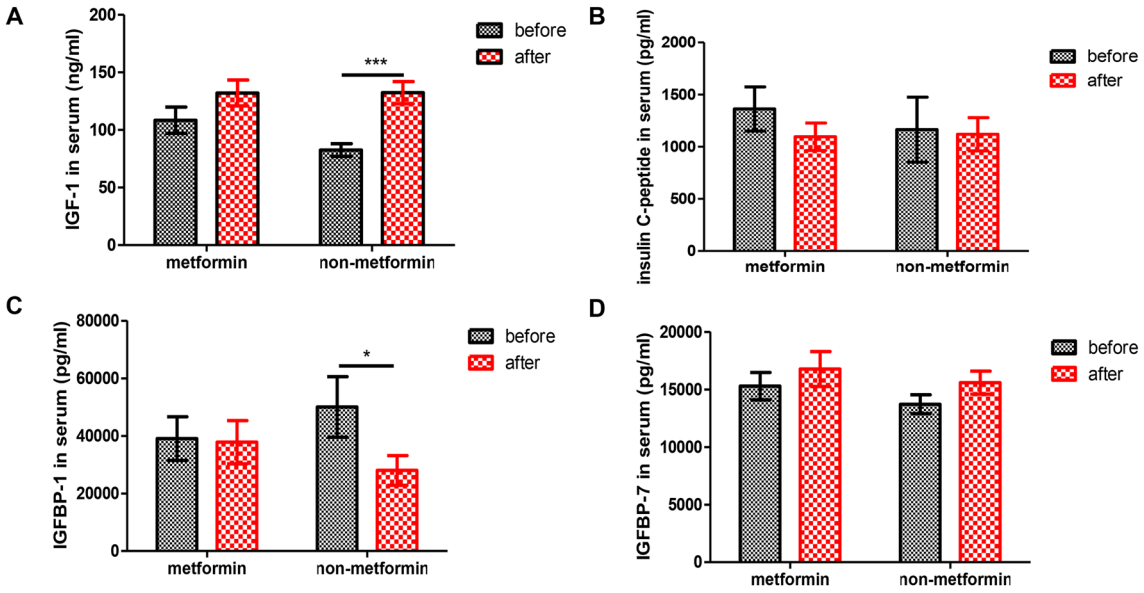

Fig. 5 The changes of IGF-1, insulin C-peptide, IGFBP-1 and IGFBP-7 in patients serum after metformin intake or not. Paired *t*-test was used to compare the changes of IGF-1, insulin C-peptide, IGFBP-1 and IGFBP-7 in serum in metformin group and control group. $*p < 0.05$, $***p < 0.001$

difference between the two groups. Intriguingly, BMI was found to be associated with prognosis of epithelial ovarian cancer patients, which was consistent with previous studies [8, 31] although *p* values were above 0.05. The PFS and DFS of patients with overweight or obese were 6 months and 4 months shorter than those with normal weight, respectively. What is more, metformin maintained the level of IGFBP-1 and inhibited the increase of IGF-1 obviously. The above evidence suggested that metformin indeed regulated the systemic insulin/IGF pathway. In our previous study, metformin inhibited the proliferation and migration of ovarian cancer cells and activated the AMPK/mTOR signaling pathway in vivo which enhanced the tumor inhibition effect of cisplatin [23, 32].

In prospective clinical studies on ovarian cancer, the ovarian cancer tissues after interventions could not be acquired; therefore, molecules on AMPK/mTOR signaling pathway could not be compared before and after interventions. As mentioned above, insulin resistance was associated with poor prognosis of various malignances. In the current study, only BMI was collected and analyzed because the HOMA index (Homeostasis model assessment index), an indicator of insulin sensitivity, could not be calculated which was one of the limitations of our study. Also, the small sample size and short follow-up may contribute to the insignificant survival benefit. Many participants in the study have not recurred till now and we will continue to follow-up these patients and analyze the PFS and overall survival (OS). More participants and a longer follow-up visit are demanded. In addition, short-term and long-term toxic and side effects should be traced and recorded.

In conclusion, within the limitations of the small sample size, there was no evidence of meaningful effect on PFS by metformin even though evidence of modulation of IGF-1 signaling axis was apparent. Large prospective, randomized clinical studies with longer follow-up period are required to confirm the effect of metformin on the prognosis of epithelial ovarian cancer.

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Compliance with ethical standards

Conflict of interest No potential conflict of interest relevant to this article was reported.

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