Association between preoperative serum insulin levels and lymph node metastasis in endometrial cancer—a prospective cohort study

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Abstract
Endometrial cancer is a common gynecological malignancy in developed countries. Insulin has been identified as a risk factor for endometrial cancer. However, whether insulin levels are related to the risk of lymph node metastasis (LNM) in endometrial cancer is unknown. We conducted a prospective cohort study in a regional hospital to examine the relationships between insulin levels and risk of LNM in premenopausal and postmenopausal women. A total of 668 patients were recruited. Of these, 206 were premenopausal (mean age: 42.01 ± 10.17) and 462 were postmenopausal (mean age: 62.13 ± 13.85). The incidence of LNM in both premenopausal and postmenopausal groups was comparable at 7% and 8%, respectively. In premenopausal women, multivariate logistic regression demonstrated that insulin levels (OR: 2.11, 95% CI: 1.48–2.85, P < 0.05) were significant predictors of LNM risk. In the same group, insulin levels remained significant predictors of LNM risk (cut-off: 10.48 μIU/mL) when adjusted for body mass index (BMI) (OR: 3.51, 95% CI: 1.42–5.98; P < 0.05) or for waist-to-hip ratio (WHR) (cut-off: 10.15 μIU/mL, OR: 1.61, 95% CI: 1.04–2.35; P < 0.05). Similarly, in postmenopausal women, multivariate logistic regression showed that insulin levels (OR: 1.99, 95% CI: 1.30–2.89; P < 0.05) also significantly predicted LNM risk. This relationship was maintained even after adjustment for BMI (cut-off: 7.40 μIU/mL, OR: 1.99, 95% CI: 1.01–3.12, P < 0.05) or for WHR (cut-off: 10.15 μIU/mL, OR: 1.61, 95% CI: 1.04–2.35; P < 0.05). Insulin levels are significantly associated with LNM risk in both premenopausal and postmenopausal women with endometrial cancer. Further prospective studies are needed to examine a potential causal relationship and determine whether its use can offer incremental value for risk stratification in this patient population.

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Introduction
Endometrial cancer is a common gynecological malignancy in developed countries. In 2017, an estimated 61,380 newly diagnosed cases and 10,920 deaths occurred due to this condition in the United States alone [1]. About 85% of cases are endometrioid adenocarcinoma, which is characterized by a slow development process and a relatively good prognosis [2]. Since symptoms such as abnormal vaginal bleeding usually present earlier, most patients receive their diagnoses at an early stage [3]. Moreover, in approximately 75% of patients, the malignancy is confined to the uterus at the time of the diagnosis [4]. Pelvic and para-aortic lymph node metastasis has been observed...
in 9% and 5% of affected patients, respectively [5, 6]. According to International Federation of Gynecology and Obstetrics (FIGO) staging system, this lymphatic involvement often indicates a poor prognosis [7].

To date, surgery is the primary treatment for endometrial cancer. According to the FIGO 2009 document [7], the operation procedure should include a hysterectomy, bilateral salpingo-oophorectomy, pelvic and para-aortic lymphadenectomy, and peritoneal cytology for staging. Except for the removal of metastatic lesions, systematic lymph node resection can decrease pelvic recurrence and provide guidance for therapeutic strategy [8, 9]. However, two randomized control trials suggest otherwise, having demonstrated a limited benefit for those who have undergone a systematic lymphadenectomy [10, 11]. The most frequently observed complications in such a procedure include ileus obstruction, extended ileus, deep venous thrombosis, and lymphocyst formation [12, 13]. At this juncture, there is little consensus regarding the routine use of lymphadenectomy in the treatment of endometrial cancer. Furthermore, although there is no singular definitive method for evaluating the extent of lymph node metastasis preoperatively, methods including magnetic resonance imaging, computed tomography, and B-mode ultrasonography all possess diagnostic value [14]. However, these diagnostic methods are only moderately successful, thereby necessitating the need for additional biomarker testing, such as the examination of serum cancer antigen-125 (Ca-125) levels, which may, in turn, provide incremental value for risk stratification. Unfortunately, this is neither a sufficiently sensitive test, as only a small proportion of endometrial cancer patients have increased Ca-125 levels [15], nor a specific test, as Ca-125 levels are also elevated in other conditions such as inflammatory diseases and atrial fibrillation [16].

More recent studies have focused on the role of metabolic parameters in aiding risk stratification in endometrial cancer [17], as it is an estrogen-dependent malignancy. Insulin resistance has been identified as a significant risk factor [18] for endometrial cancer. Insulin resistance refers to a condition in which target organs show a reduced responsiveness to insulin. This results in a hyperactivity of pancreatic cells, which attempt to compensate for the attenuated sensitivity, leading to hyperinsulinemia [19]. Epidemiological evidence has shown a correlation between insulin resistance and endometrial cancer. The mechanisms supporting this association can be explained primarily through both the direct and indirect roles of insulin in endometrial cancer development. Directly, insulin binding to its cognate receptor induces the activation of the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/Akt and Ras/mitogen-activated protein kinase (MAPK) pathways, which trigger the activation of proteins involved in cell cycle regulation. With cancer, signaling in these pathways is often dysfunctional and hyperactive [20], and can in turn precipitate in cell proliferative and anti-apoptotic effects that collectively promote endometrial cancer development [18]. Insulin indirectly induces the pathogenesis of endometrial cancer by inhibiting the synthesis of sex hormone-binding globulin (SHBG), which normally binds to androgens and estrogens. With hyperinsulinemia, this inhibition, in turn, increases the levels of free androgens (which can then be converted to estrogens in peripheral tissues) and estrogens. As suggested by the “unopposed estrogen” hypothesis, without sufficient protection from progesterone, the resulting elevated plasma estrogen levels induce cell proliferation in the endometrium, thereby promoting carcinogenesis [21]. The other possible mechanistic links between insulin and endometrial cancer will be further elaborated upon later.

Our group has reported that insulin was associated with a dose-dependent increase in endometrial cancer risk [21]. However, there have been little data on the association between insulin levels and the risk of lymph node metastasis. Therefore, we conducted a case–control study in China to evaluate whether insulin is a preoperative indicator of lymph node metastasis in endometrial cancer.

Subjects and Methods

Patient selection

This study was approved by the Local Ethics Committee of Yantai Yuhuangding Hospital in Shandong, China (Approval Number: E2009033). Consecutive patients with a histological diagnosis of endometrial cancer between January 2010 and December 2016 at the Yantai Yuhuangding Hospital in Shandong, China, were enrolled. All subjects provided written informed consent. Exclusion criteria included the use of steroid hormones within the past 12 months of the study.

A total of 668 patients, including 206 premenopausal and 462 postmenopausal women, were included in our study. All patients received standard staging operations including a systematic lymphadenectomy. All operations were performed by the same surgeons. All specimens were diagnosed histologically by two independent, experienced pathologists. Patients were divided according to menopause status into a premenopausal group and a postmenopausal group. Within each group, patients were further divided into a lymph node metastasis (LNM) group and a non-lymph node metastasis (NLNM) group.

Anthropometric measurements and history taking

All anthropometric measurements were performed on their first day of admission to our hospital. Each measurement
was performed twice, and the mean value was then calculated. The history obtained from the patients included reproductive history, medical history, and family history of malignancy.

**Collection and storage of blood samples**

On the morning of the operation, 5 mL of venous blood was drawn from each patient. The blood samples were delivered to the laboratory immediately for centrifugation. The serum samples were stored at −80°C for future analysis.

**Laboratory examinations**

The serum samples were assayed at the clinical laboratory of Yantai Yuhuangding Hospital. The laboratory staff was blinded to the status of the patients. As the estrogen level of premenopausal women depends on the phase of the menstrual cycle, estrogen level was only determined in postmenopausal women. Serum insulin and estrogen concentrations were examined by radioimmunoassay (Siemens Medical Solutions Diagnostics, Los Angeles, USA) which demonstrated an interassay correlation variation [CV] of 6.0% and 5.6%, respectively. Serum C-peptide level was measured using chemoluminescence (Siemens Healthcare Diagnostics Inc, Gwynedd, UK; interassay CV of 6.1%). Serum interleukin-6 (IL-6) level was measured by enzyme-linked immunoassay (R&D Systems Europe, Lille, France; interassay CV of 9.2%). Serum tumor necrosis factor (TNF)-α level was measured by a multiplex assay (Milliplex Human Adipokine Panel B, Millipore, Billerica, MA; interassay CV of 16.3%). Serum Ca-125 level was measured with an electrochemiluminescence immunoassay with ADVIA CENTAUR XP (Siemens, Munich, Germany; interassay CV of 3.9%).

**Statistical analysis**

SAS software package (Version 9, SAS Institute, Cary, NC) was used for data analysis. Serum estrogen, insulin, C-peptide, CA125, TNF-α, and IL-6 levels were all natural logarithm-transformed to normalize their distributions. Differences between groups were tested for continuous variables using the Student’s t-test and for categorical variables using the chi-square test. Spearman correlation coefficients were calculated to evaluate the correlations between insulin and potential confounders. Univariate and multivariate logistic regression models were developed to estimate the associations between the variables and LNM risk. Serum insulin levels of pre- and postmenopausal patients were categorized into quartiles, with cutoff points determined based on the distributions of the patients with NLNM. Likelihood ratio tests were performed to examine the linear trends in ORs with assigned quantitative scores, 1, 2, 3, and 4 for the categories. As obesity, particularly visceral obesity, is a well-established risk factor for insulin resistance, BMI and WHR were adjusted for in our analysis. All P values were two-sided among which lower than 0.05 is considered statistically significant.

**Results**

**Basic characteristics of patients with lymph node metastases and nonlymph node metastases**

A total of 668 patients were recruited into this study, whose baseline and pathological characteristics are shown in Table 1. Of these, 206 were premenopausal and 462 were postmenopausal. For the premenopausal cohort, 14 women showed evidence of LNM (7%), whereas the remaining 192 did not. Those with LNM had similar age (42.4, 95% confidence interval [CI]: 34.0–51.4 years vs. 41.8, 32.2–52.2 years; P > 0.05), a higher BMI (28.9, 22.7–34.7 kg/m² vs. 24.4, 20.2–32.1 kg/m²; P < 0.001), and WHR (0.9, 0.8–1.0 vs. 0.8, 0.7–0.9; P < 0.001), but nevertheless similar proportions of high birthweight >4 kg (21% vs. 22%) and statistically indistinguishable age at menarche (14.0, 9.8–17.0 years vs. 13.6, 10.4–17.0 years) compared to those with NLNM. The incidences of diabetes (36% vs. 24%, P > 0.05) and hypertension (27% vs. 16%, P > 0.05) were not significantly different between both groups. However, the LNM group had a higher proportion of patients with lesion diameter ≥2 cm (57% vs. 19%; P < 0.01) and with myometrial invasion ≥50% (43% vs. 14%; P < 0.05). The LNM group also had a lower proportion of patients with pathological grade 1 (29% vs. 81%; P < 0.001) and a higher proportion with pathological grade 2 (29% vs. 9%; P < 0.05) and 3 (43% vs. 10%; P < 0.01) lesions. Finally, patients with LNM had higher Ca-125 (22.3, 9.4–322.8 U/mL vs. 14.3, 4.7–299.2 U/mL; P < 0.05) and insulin (11.0, 8.4–14.1 μU/mL vs. 7.9, 3.2–12.2 μU/mL; P < 0.01) levels but statistically indistinguishable CRP (1205, 1161–1488 ng/mL vs. 1291, 1029–1379 ng/mL; P > 0.05), TNF-α (1.1, 0.9–1.7 pg/mL vs. 1.1, 0.7–1.8 pg/mL; P > 0.05), and IL-6 (1.5, 1.1–1.7 pg/mL vs. 1.5, 1.2–1.7 pg/mL; P > 0.05) levels compared to those with NLNM.

For the postmenopausal cohort, 35 women showed evidence of LNM (8%), whereas the remaining 427 did not. Those with LNM were older (64.7, 49.5–76.0 years vs. 59.2, 50.1–74.7 years; P < 0.05), had a higher BMI (28.2, 20.8–33.6 kg/m² vs. 25.0, 20.6–32.0 kg/m²; P < 0.001) and WHR (0.9, 0.8–1.0 vs. 0.8, 0.7–0.9;
but nevertheless similar proportions of high birthweight >4 kg (25% vs. 24%; \( P > 0.05 \)) and statistically indistinguishable age at menarche (14.5, 10.0–15.1 years vs. 14.6, 10.5–16.1 years; \( P > 0.05 \)) and age at menopause (51.7, 46.3–56.4 years vs. 50.5, 47.3–54.9 years; \( P > 0.05 \)) compared to those with NLNM.

The incidences of diabetes (29% vs. 26%; \( P > 0.05 \)) and hypertension (37% vs. 47%; \( P > 0.05 \)) were not significantly different between both groups. However, the LNM group had a higher proportion of patients with lesion diameter >2 cm (49% vs. 15%; \( P < 0.001 \)) and with myometrial invasion ≥50% (46% vs. 9%; \( P < 0.01 \)). The LNM group also had a lower proportion with pathological grade 1 (9% vs. 83%; \( P < 0.001 \)) and a higher proportion with pathological grade 2 (26% vs. 13%; \( P < 0.05 \)) and 3 (66% vs. 4%; \( P < 0.001 \)) lesions. Finally, patients with LNM had higher Ca-125 (22.36 (9.42–322.84) vs. 14.29 (4.73–299.17); \( P < 0.001 \)) and Estrogen (pg/mL) – – – (23.97 (13.85–40.99)) vs. 21.01 (9.03–36.48) (0.061) compared to those with NLNM. Insulin levels positively correlated with estrogen levels, Table 1.

Table 1. Clinical and pathological characteristics of LNM and NLNM endometrial cancer patients.

|                         | Premenopausal women                      | Postmenopausal women                      |
|-------------------------|------------------------------------------|-------------------------------------------|
|                         | LNM (n = 14)                             | LNM (n = 35)                              | NLNM (n = 192)                           | NLNM (n = 427)                           |
|                         |                                          |                                          |                                          |                                          |
| Age                     | 42.43 (34.01–51.39) vs. 41.84 (32.17–52.18) | 64.72 (49.51–76.02) vs. 59.20 (50.05–74.67) | 0.339                                     | 0.048                                     |
| BMI (Kg/m²)             | 28.94 (22.73–34.69) vs. 24.38 (20.19–32.09) | 28.15 (20.76–33.58) vs. 25.01 (20.64–32.01) | <0.001                                    | <0.001                                    |
| WHR                     | 0.91 (0.80–0.99) vs. 0.80 (0.69–0.93)     | 0.88 (0.75–0.96) vs. 0.81 (0.67–0.94)     | <0.001                                    | <0.001                                    |
|Birthweight >4 kg        | 3 (21.42) vs. 4 (21.88)                  | 9 (25.71) vs. 104 (24.36)                | 0.969                                     | 0.083                                     |
| Age at menarche         | 13.98 (9.81–17.01) vs. 13.56 (10.44–17.03) | 14.54 (10.00–15.09) vs. 14.57 (10.52–16.06) | 0.779                                     | 0.862                                     |
| Age at menopause        | –                                        | –                                        | –                                        | 0.997                                     |
| Diabetes (%)            | 5 (35.71) vs. 46 (23.96)                | 10 (28.57) vs. 113 (26.46)               | 0.342                                     | 0.843                                     |
| Hypertension (%)        | 4 (26.67) vs. 30 (15.63)                | 13 (37.14) vs. 201 (47.07)               | 0.278                                     | 0.293                                     |
| Lesion diameter >2 cm   | 8 (57.14) vs. 36 (18.75)                | 17 (48.57) vs. 66 (15.46)                | 0.003                                     | <0.001                                    |
| Myometrial invasion ≥50% (%) | 6 (42.86) vs. 27 (14.06) | 16 (45.71) vs. 39 (9.13) | 0.013                                     | 0.002                                     |
| Pathologic grade (%)    |                                          |                                          |                                          |                                          |
| 1                       | 4 (28.57) vs. 155 (80.73)                | 3 (8.57) vs. 354 (82.90)                 | <0.001                                    | <0.001                                    |
| 2                       | 4 (28.57) vs. 18 (9.37)                 | 9 (25.71) vs. 55 (12.88)                | 0.048                                     | 0.043                                     |
| 3                       | 6 (42.86) vs. 19 (9.90)                 | 23 (65.72) vs. 18 (4.22)                | 0.003                                     | <0.001                                    |
| Ca-125 (U/mL)           | 22.36 (9.42–322.84) vs. 14.29 (4.73–299.17) | 24.18 (7.46–376.33) vs. 12.69 (3.34–215.64) | 0.021                                     | 0.019                                     |
| Estrogen (pg/mL)        | –                                        | –                                        | 23.97 (13.85–40.99) vs. 21.01 (9.03–36.48) | 0.061                                     |
| Insulin (µIU/mL)        | 11.01 (8.42–14.06) vs. 7.91 (3.17–12.19) | 10.59 (8.08–14.55) vs. 7.13 (2.99–13.22) | 0.008                                     | 0.011                                     |
| CRP (ng/mL)             | 1205 (1161–1488) vs. 1291 (1029–1379)    | 1176 (901–1354) vs. 1125 (821–1466)      | 0.953                                     | 0.899                                     |
| TNF-α (pg/mL)           | 1.07 (0.88–1.69) vs. 1.05 (0.67–1.78)    | 1.06 (0.79–1.69) vs. 1.06 (0.55–1.57)    | 0.716                                     | 0.964                                     |
| IL-6 (pg/mL)            | 1.48 (1.11–1.69) vs. 1.49 (1.22–1.70)    | 1.42 (1.09–1.77) vs. 1.41 (1.01–2.13)    | 0.822                                     | 0.901                                     |

LNM, lymph node metastasis; NLNM, nonlymph node metastasis; BMI, body mass index; WHR, waist-to-hip ratio; CRP, C-reactive protein.

Continuous variables are shown as means (95% CI), while categorical variables are shown as their positive percentages.

Correlations between clinical or biochemical parameters and insulin levels in endometrial cancer patients

The correlations between clinical or biochemical parameters and insulin levels are shown in Table 2. In the LNM and NLNM groups of both premenopausal and postmenopausal women, insulin levels showed significant positive correlations with BMI and WHR but not with high birthweights, age at menarche, or age at menopause. Moreover, insulin levels also showed positive correlations with diabetes in premenopausal and postmenopausal LNM and NLNM groups, but the correlation coefficient for the postmenopausal LNM group did not reach statistical significance (\( P > 0.05 \)). Insulin levels further showed positive correlations with hypertension in premenopausal and postmenopausal LNM groups, but not in NLNM groups. Insulin levels were significantly and positively correlated with greater disease severity of increased lesion diameter >2 cm, myometrial invasion ≥50%, and pathological grade. Finally, insulin levels positively correlated with estrogen levels, 1.1–1.8 pg/mL vs. 1.4, 1.0–2.1 pg/mL; \( P > 0.05 \)) levels compared to those with NLNM.
but not with Ca-125, CRP, TNF-α, or IL-6 in any of the groups.

**Predictors of lymph node metastases**

Univariate logistic regression was used to examine the relationship between clinical or biochemical parameters and LNM risk. In premenopausal women (Table 3), BMI, WHR, lesion diameter >2 cm, myometrial invasion ≥50%, and pathological grade, but not age, high birthweight, age at menarche, diabetes, or hypertension, were associated with LNM risk. In terms of biochemical parameters, Ca-125, and insulin, but not CRP, TNF-α or IL-6 predicted LNM risk. Stepwise multivariate logistic regression then demonstrated that only WHR, lesion diameter ≥2 cm, myometrial invasion ≥50%, pathological grade, and insulin levels remained significant predictors of LNM risk. The same parameters that showed significant predictive values

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**Table 2.** Spearman correlation coefficients for associations between circulating insulin levels and the risk factors of lymph node metastasis.

|                      | LNM (n=14) | NLNM (n=192) | LNM (n=35) | NLNM (n=427) |
|----------------------|------------|--------------|------------|--------------|
| **Insulin (premenopausal women)** |            |              |            |              |
| Age                  | 0.05       | 0.09         | 0.19†      | 0.17         |
| BMI                  | 0.41†      | 0.37‡        | 0.39‡      | 0.42‡        |
| WHR                  | 0.31†      | 0.33‡        | 0.33‡      | 0.40‡        |
| Birthweight >4 kg    | 0.11       | 0.15         | 0.16       | 0.14         |
| Age at menarche      | 0.06       | 0.13         | 0.12       | 0.09         |
| Age at menopause     | –          | –            | 0.14       | 0.12         |
| Diabetes             | 0.18†      | 0.19†        | 0.17       | 0.19†        |
| Hypertension         | 0.20†      | 0.16         | 0.22†      | 0.15         |
| Lesion diameter >2 cm| 0.24†      | 0.22†        | 0.27†      | 0.19†        |
| Myometrial invasion ≥50% | 0.29†   | 0.30†        | 0.31†      | 0.31†        |
| Pathologic grade     | 0.33‡      | 0.32‡        | 0.30†      | 0.28†        |
| Ca-125               | 0.14       | 0.16         | 0.17       | 0.15         |
| Estrogen             | –          | –            | 0.27†      | 0.25†        |
| CRP                  | 0.09       | 0.14         | 0.11       | 0.09         |
| TNF-α                | 0.11       | 0.12         | 0.13       | 0.10         |
| IL-6                 | 0.15       | 0.14         | 0.14       | 0.13         |

**Table 3.** Odds ratio for lymph node metastasis risk among premenopausal endometrial cancer patients using univariate and multivariate logistic regressions.

|                               | Univariate logistic regression |          |          |          |          |
|-------------------------------|-------------------------------|----------|----------|----------|----------|
|                               | OR 95% CI                      | P value  | OR 95% CI | P value  |
| Age                           | 0.88 0.52–1.28                | 0.195    | 0.75 0.44–1.20 | 0.223    |
| BMI                           | 2.28 1.79–2.80                | 0.031    | 1.51 0.93–1.90 | 0.059    |
| WHR                           | 4.94 2.52–11.56               | 0.003    | 2.59 1.36–3.81 | 0.035    |
| Birthweight >4 kg             | 1.23 0.63–1.99                | 0.453    | 1.02 0.55–1.67 | 0.637    |
| Age at menarche               | 1.51 0.98–2.18                | 0.297    | 1.07 0.47–1.64 | 0.258    |
| Diabetes                      | 1.88 0.72–2.26                | 0.297    | 1.16 0.42–1.82 | 0.452    |
| Hypertension                  | 1.54 0.65–2.30                | 0.095    | 1.22 0.64–1.71 | 0.106    |
| Lesion diameter >2 cm         | 2.67 1.56–3.88                | 0.033    | 1.59 1.20–1.87 | 0.040    |
| Myometrial invasion ≥50%      | 4.22 2.67–6.06                | 0.002    | 2.64 1.99–4.23 | 0.009    |
| Pathological grade            | 4.30 2.77–6.84                | 0.004    | 2.43 1.95–3.16 | 0.008    |
| Ca-125                        | 2.71 1.20–2.89                | 0.038    | 1.84 0.97–3.10 | 0.057    |
| Insulin                       | 3.32 1.88–4.69                | 0.007    | 2.11 1.48–2.85 | 0.023    |
| CRP                           | 1.88 0.94–2.79                | 0.089    | 1.09 0.74–1.51 | 0.196    |
| TNF-α                         | 1.96 0.90–3.21                | 0.079    | 1.28 0.75–1.92 | 0.109    |
| IL-6                          | 1.53 0.76–2.46                | 0.081    | 1.06 0.46–1.72 | 0.202    |
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for LNM risk in both univariate and multivariate analyses in premenopausal women were also predictive in postmenopausal women (Table 4).

Subsequent analyses involved the categorization of insulin levels into quartiles. In premenopausal women (Table 5), compared to the first quartile, there was a dose-dependent relationship between insulin levels and LNM risk with odds ratios of 1.51 (95% CI: 0.90–2.49; \( P > 0.05 \)), 2.23 (1.34–3.39; \( P < 0.05 \)), and 4.88 (2.26–7.05; \( P < 0.05 \)) for the second, third, and fourth quartiles, respectively. However, only quartile four (cut-off: 10.48 \( \mu \)IU/mL) remained predictive of LNM risk when adjusted for BMI (OR: 3.51, 1.42–5.98; \( P < 0.05 \)) or for WHR (OR: 1.87, 1.08–2.66; \( P < 0.05 \)).

Discussion

The main findings of this study are that (1) WHR, lesion diameter >2 cm, myometrial invasion ≥50%, pathological grade, and insulin levels were significant predictors of LNM risk in both premenopausal and postmenopausal women, (2) insulin level with a cut-off of 10.48 \( \mu \)IU/mL was predictive of LNM risk when adjusted for BMI (OR: 3.51, 1.42–5.98; \( P < 0.05 \)) or for WHR (OR: 1.87, 1.08–2.66; \( P < 0.05 \)).

Table 4. Odds ratio for lymph node metastasis risk among postmenopausal endometrial cancer patients using univariate and multivariate logistic regressions.

| Categories                            | Univariate logistic regression | Multivariate logistic regression |
|---------------------------------------|--------------------------------|--------------------------------|
|                                       | OR 95% CI                      | OR 95% CI                      |
| Age                                   | 0.97 0.49–1.58 0.099           | 0.86 0.56–1.29 0.218           |
| BMI                                   | 2.72 1.29–5.01 0.039           | 1.62 0.93–2.46 0.054           |
| WHR                                   | 5.07 2.33–7.63 0.002           | 2.21 1.29–3.64 0.022           |
| Birthweight >4 kg                     | 1.43 0.79–1.64 0.204           | 1.11 0.64–1.76 0.532           |
| Age at menarche                       | 1.21 0.39–2.06 0.752           | 0.94 0.30–1.61 0.737           |
| Age at menopause                      | 1.34 0.46–2.39 0.684           | 0.85 0.24–1.55 0.691           |
| Diabetes                              | 1.90 0.81–3.21 0.167           | 1.34 0.64–2.20 0.533           |
| Hypertension                          | 1.57 0.71–2.24 0.678           | 1.11 0.55–1.83 0.672           |
| Lesion diameter >2 cm                 | 2.44 1.51–3.56 0.019           | 1.68 1.37–3.09 0.038           |
| Myometrial invasion ≥50%              | 4.98 2.97–6.84 0.011           | 2.41 1.39–3.62 0.027           |
| Pathologic grade                      | 5.30 2.47–8.20 <0.001          | 2.52 1.77–3.23 0.012           |
| Ca-125                                | 2.04 1.31–3.33 0.031           | 1.71 0.98–2.85 0.052           |
| Estrogen                              | 1.85 0.97–2.94 0.054           | 1.29 0.78–1.65 0.072           |
| Insulin                                | 3.91 2.06–5.34 <0.001          | 1.99 1.30–2.89 0.029           |
| CRP                                   | 1.34 0.64–1.96 0.093           | 1.01 0.52–1.68 0.124           |
| TNF-\( \alpha \)                      | 1.53 0.76–2.22 0.097           | 1.08 0.67–1.55 0.089           |
| IL-6                                   | 1.34 0.71–2.03 0.073           | 0.97 0.51–1.45 0.103           |

Table 5. Risk (odds ratio, OR (95% confidence interval, CI)) of lymph node metastasis risk among premenopausal endometrial cancer patients by categories of insulin.

| Categories | 1 | 2 | 3 | 4 | \( P_{\text{trend}} \) |
|------------|---|---|---|---|------------------|
| Quartile cut-offs (\( \mu \)IU/mL) | \(<5.56\) | 5.56–7.64 | 7.65–10.48 | >10.48 | \( P_{\text{trend}} \) |
| Crude OR   | 1 | 1.51 (0.90–2.49) | 2.23 (1.34–3.39) | 4.88 (2.26–7.05) | 0.034 |
| Adjusted for BMI | 1 | 1.34 (0.66–2.19) | 2.04 (0.95–3.26) | 3.51 (1.42–5.98) | 0.042 |
| Adjusted for WHR | 1 | 1.02 (0.52–1.65) | 1.89 (0.84–2.86) | 1.87 (1.08–2.66) | 0.048 |

\( P_{\text{trend}} \) value for trend with assigned quantitative scores 1, 2, 3, and 4 for the categories. Cut-off points were based on the distribution of the nonlymph node metastasis premenopausal patients.
P < 0.05) in premenopausal women, and (3) insulin with a similar cut-off of 10.15 μIU/mL was predictive of LNM risk when adjusted for BMI (3.07, 1.26–5.40; P < 0.05, respectively) or for WHR (OR: 1.61, 1.04–2.35; P < 0.05).

In our previous case–control study conducted in China [21], we identified insulin as an independent predictor of endometrial cancer in premenopausal women. Similarly, a case–control study involving a Caucasian population in Alberta, Canada, also found that the highest quartile of insulin, in comparison with the lowest quartile, was associated with an increased risk of endometrial cancer [22]. In another prospective cohort study, insulin levels, comparing the highest with the lowest quartile, were associated with a twofold increase in the risk of endometrial cancer in 93,676 postmenopausal women [23]. We extend these findings by demonstrating that insulin was an independent predictor of LNM risk in endometrial cancer for both premenopausal and postmenopausal Chinese women.

Epidemiological studies have shed insights into a possible mechanistic link between insulin and the risk of endometrial cancer [24], which includes sex steroid hormones, adipokines, and low-grade inflammation. Firstly, endometrial cancer is an estrogen-dependent cancer [25] and as aforementioned, insulin can inhibit the synthesis of sex hormone-binding globulin (SHBG) that usually binds to steroid hormones [26], leading to increased free estrogen levels in the blood. Estrogen exerts its effects by stimulating the ERα, ERβ, or GPER (G protein-coupled estrogen receptor 1) receptors, leading to a proliferative response in the endometrium [27]. Secondly, as also stated previously, insulin may act independently via its downstream PI3K/AKT and Ras/MAPK signaling pathways [28] to induce cell proliferation and inhibit apoptosis, thereby promoting endometrial cancer development. Thirdly, insulin can increase the circulating levels of insulin growth factor-1 [29], which has been linked to an increased proliferation of the endometrium. Fourthly, increased insulin levels are associated with obesity, and several adipokines have in turn been implicated in the disease pathogenesis of endometrial cancer. Thus, in a case–control study within the Prostate, Lung, Colorectal, and Ovarian Cancer (PLCO) Screening Trial, the relationship of prediagnostic serum levels of adiponectin, leptin, and visfatin with postmenopausal endometrial cancer risk was prospectively evaluated [30]. This demonstrated that higher leptin levels were associated with higher cancer risk, whereas higher adiponectin levels and adiponectin-to-leptin ratio were associated with a lower risk. Finally, there is a relationship between insulin levels, insulin resistance, chronic inflammation, and endometrial cancer risk [31, 32]. Low-grade inflammation can promote the neoplastic transformation of endometrial tissue via several mechanisms, namely by inducing the generation of reactive oxygen species, which causes DNA mutations and increased cell proliferation [33, 34], as well as by mediating the dysregulation of the NF-κB pathway, leading to the inhibition apoptosis [35].

Conclusion
In conclusion, this epidemiological study demonstrated insulin to be an independent predictor of LNM. Further prospective studies are needed to confirm this association and its role as a biomarker for determining clinical outcomes and prognosis. Finally, basic science studies are needed to elucidate the mechanistic pathways that potentially explain a causal relationship between insulin levels and cancer metastasis in endometrial cancer.

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Conflict of Interest
None declared.
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