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Research Paper

Impact of Estrogen on the Relationship Between Obesity and Renal Cell Carcinoma Risk in Women

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ABSTRACT

The relationship between obesity and renal cell carcinoma (RCC) has been widely investigated. However, the effect of estrogen on this relationship in female RCC patients has not been evaluated. We conducted a case-control study to investigate the role of estrogen as a potential modifier of the association between obesity and RCC risk in Chinese women.

A total of 497 consecutive female patients with pathologically confirmed RCC, including 364 clear cell RCC (ccRCC), were enrolled. Age-matched controls were selected from cancer-free females seeking physical examination in our institution. Estrogen receptor-β (ER-β) and insulin-like growth factor (IGF)-1 receptor (IGF-1R) expression levels were detected in RCC tissues. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were estimated by logistic regression models.

We observed a positive association between overweight and RCC risk in pre-menopausal but not post-menopausal women. Similar association was also observed between overweight and ccRCC risk. Overweight pre-menopausal women had an increased risk of RCC (OR: 1.67, 95%CI: 1.01–2.76), as well as an increased risk of ccRCC (OR: 1.73, 95%CI: 1.02–2.99), after adjusting for potential confounders. IGF-1R expression levels were higher in pre-menopausal compared with post-menopausal cases ($P = 0.015$).

These results suggest that estrogen plays an important role in RCC etiology and may modify the association between obesity and RCC risk in women. We hypothesize that estrogen may up-regulate IGF-1R and potentiate the deleterious effects of obesity-related elevations of insulin and IGFs.

1. Introduction

Renal cell carcinoma (RCC) is the most common kidney-derived malignancy, and accounts for 2%–3% of all adult cancers [4]. RCC has increased in incidence in recent decades, and is usually diagnosed in patients aged between 50 and 70 years [20]. Genetic background is thought to be a pivotal factor in RCC carcinogenesis; for example, approximately two-thirds of patients with clear cell RCC (ccRCC) harbor mutations in the von Hippel Lindau gene [1], while loss of chromosome 3p is also prevalent in ccRCC [15]. In contrast, environmental factors such as a Western lifestyle, including excess energy intake and minimal physical activity leading to obesity and hypertension [2,5], are also important contributory factors to RCC etiology.

The relationship between obesity and RCC risk has been widely investigated. Obesity, indicated by a high body mass index (BMI), is associated with increased long-term RCC risk [2,12] and unfavorable oncological outcomes of RCC [3]. Furthermore, obesity can promote a cascade of secondary metabolic pathologies, such as hypertension, diabetes, and dyslipidemia. These pathologies, either alone or in combination, may exacerbate the development of RCC via complex pathways involving insulin resistance, adipokines, inflammation, and other important molecular mechanisms. Many crucial signaling molecules, including the insulin-like growth factor (IGF) axis, adiponectin, and hypoxia-inducible factors, function as mediators between obesity and RCC [29,31].

Several aspects of RCC show remarkable gender differences. First, the incidence of RCC is higher in men than in women, with an approximate ratio of 2:1 [20]. Second, patients with papillary RCC are less likely to be female while chromophobe RCC patients are more likely to be female, compared with ccRCC as the major histological subtype of RCC [13]. Third, analysis of a large database found that younger female RCC

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patients (pre-menopausal) showed lower renal cancer-specific mortality in relation to both localized and advanced diseases compared with their male counterparts, though the sex disparities for localized and advanced RCC were diminished and even reversed, respectively, in post-menopausal women [17]. Although the reasons for these differences remain unclear, it has been supposed that sex hormones might play an important role in RCC development and in the observed disparities.

Body fat is correlated with altered insulin levels and with sex hormone secretion and storage. Previous studies reported the co-regulation of sex steroids and insulin, mainly in breast cancer [6,11]. Recent studies of the modifying effect of estrogen status on the influence of obesity on colorectal neoplasm risk [21] found that the association between obesity and colorectal cancer risk in women was limited to certain subgroups, based mostly on their estrogen status [8]. Obesity was only associated with increased colorectal adenoma risk among pre-menopausal women [27]. However, the role of estrogen status in the association between obesity and RCC risk in women has not yet been investigated. We conducted a multicenter case-control study to explore the role of estrogen as a potential modifier of the relationship between obesity and RCC risk in Chinese women. We also studied the co-regulation of estrogen and the IGF axis in RCC tissues from women to evaluate the mechanisms whereby estrogen may influence the observed association between obesity and RCC.

2. Materials and Methods

2.1. Ethics

This study was approved by the Institutional Review Board of The Affiliated Hospital of Qingdao University. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from all subjects prior to participation.

2.2. Study Subjects

This retrospective study included 497 consecutive female patients recruited from five regional medical centers in Eastern and Northern China (The Affiliated Hospital of Qingdao University, Songshan Hospital of Qingdao University, Fudan University Shanghai Cancer Center, The Second Affiliated Hospital of Xi'an Jiaotong University, and Baotou City Central Hospital) between January 2014 and December 2014. All patients had RCC pathologically confirmed by either renal surgery or core biopsy. Age-matched controls were selected from among cancer-free females undergoing physical examination at The Affiliated Hospital of Qingdao University during 2014. Data collection included age, height, weight, waist circumference (WC), history of hypertension and diabetes, menstrual status, use of hormone replacement therapy (HRT), histological subtype, stage at diagnosis, and Fuhrman grade. Women for whom information on menstrual status was missing or unclear were excluded from the study. A total of 445 RCC cases and 508 controls were included in the analysis.

BMI was defined as the subject's weight (kg) divided by their height (m^2). BMI was categorized based on World Health Organization criteria, and a BMI ≥25 kg/m^2 was considered as overweight.

2.3. Immunohistochemistry (IHC)

We obtained paraffin-embedded ccRCC specimens from the Department of Pathology for 93 ccRCC cases from The Affiliated Hospital of Qingdao University. IHC staining was carried out as described previously [30]. Briefly, fresh tissues were fixed in formalin and embedded in paraffin, then sectioned at a thickness of 5 μm. The sections were dewaxed in xylene and rinsed in alcohol and in graded alcohol/water mixtures. Hydrogen peroxide (3%) was applied to block the endogenous peroxidase activity. The sections were subsequently treated in a microwave oven twice for 6 min in citrate buffer (pH 6.0) at 600 W to undergo antigen repairing. After blocking with goat serum for 30 min, sections were incubated with primary antibodies against estrogen receptor-β (ER-β) (ab3576) (1:50) and IGF-1R (ab39398) (1:100) (Abcam, Cambridge, MA, USA) at 4 °C overnight, followed by anti-mouse/rabbit horseradish peroxidase-labeled antibody (Univ-bio, Shanghai, China) as the second antibody. ER-β and IGF-1R staining were scored as 0, 1, 2, and 3 according to the proportion of positively stained cells and the intensity of the staining, as described previously [23].

2.4. Statistical Analysis

Continuous variables were expressed as mean ± standard deviation and were compared between groups by using Student's *t*-tests. Categorical variables were expressed as frequencies and percentages and were compared using χ^2 tests. Unconditional logistic regression analysis was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs). All statistical analyses were carried out using STATA 12.0, and two-sided P values <0.05 were considered to indicate statistical significance.

3. Results

The baseline characteristics of the 445 enrolled female RCC patients (median age, 55 years; range, 15–83 years) and 508 cancer-free female controls (median age, 53 years; range, 19–83 years) are shown in

Table 1
Clinicopathological characteristics of the 445 enrolled female RCC patients.

		Case (n = 445)	Control (n = 508)	P value
Age (mean ± SD)		53.4 ± 12.1	52.3 ± 12.5	0.928
BMI (n, %)	<25	270 (60.67)	345 (67.91)	0.020
	≥25	175 (39.33)	163 (32.09)	
Hypertension (n, %)	yes	169 (37.98)	160 (31.50)	0.036
	no	276 (62.02)	348 (68.50)	
Diabetes (n, %)	yes	131 (29.44)	85 (16.73)	<0.001
	no	314 (70.56)	423 (83.27)	
Menopause (n, %)	yes	280 (62.92)	316 (62.20)	0.820
	no	165 (37.08)	192 (37.80)	
Use of HRT (n, %)	yes	28 (6.29)	41 (8.07)	0.290
	no	417 (93.71)	467 (91.93)	
WC (n, %)	quartile1	94 (21.12)	144 (28.35)	<0.001
	quartile2	94 (21.12)	143 (28.15)	
	quartile3	130 (29.21)	111 (21.85)	
	quartile4	127 (28.54)	110 (21.65)	
Fuhrman grade (n, %)	I	22 (4.94)	/	
	II	201 (45.17)	/	
	III	128 (28.76)	/	
	IV	23 (5.17)	/	
	missing	71 (15.96)	/	
Stage at diagnosis (n, %)	I	352 (79.10)	/	
	II	55 (12.36)	/	
	III	13 (2.92)	/	
	IV	25 (5.62)	/	
Pathological type (n, %)	ccRCC	364 (81.80)	/	
	papillary RCC	18 (4.04)	/	
	chromophobe RCC	40 (8.99)	/	
	other	23 (5.17)	/	

BMI: Body Mass Index; WC: Waist Circumference; HRT: hormone replacement therapy; RCC: renal cell carcinoma; ccRCC: clear cell renal cell carcinoma.

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