

# Serum sex hormone levels in pre- and postmenopausal breast cancer patients

Ho C C K, Rohaizak M, Zulkifli S Z, Siti-Aishah M A, Nor-Aini U, Sharifah-Noor-Akmal S H

## ABSTRACT

**Introduction:** This study was conducted to determine the association between serum sex hormone levels and breast cancer.

**Methods:** The study was conducted on newly-diagnosed breast cancer patients who had not received any treatment. Controls were women not known to have any breast disease or hormone-related tumours. Serum hormones were divided into quartiles. Logistic regression adjusting for age and race were done to calculate the odds ratio (OR) and 95 percent confidence interval (CI).

**Results:** A total of 207 subjects were recruited; 73 premenopausal (37 cases, 36 controls) and 134 postmenopausal (68 cases and 66 controls) women. In the premenopausal women, only serum testosterone was positively associated with breast cancer (OR 1.72, 95 percent CI 0.40–7.40), but this was not a significant finding (p-value is 0.468). In the postmenopausal women, oestradiol, progesterone and testosterone were positively associated with breast cancer with a highest to lowest quartile OR of 1.48, 2.35 and 4.23 (95 percent CI 0.59–3.69, 1.11–4.95 and 1.52–11.78, respectively). The OR was significant for both progesterone and testosterone (p-values of 0.025 and 0.006, respectively).

**Conclusion:** There were no statistically significant findings among the premenopausal cases. In postmenopausal women, serum progesterone and testosterone levels were significantly associated positively with the odds of having breast cancer.

**Keywords:** breast cancer, hormones, postmenopausal cancer risk, progesterone, sex hormones, testosterone.

*Singapore Med J 2009;50(5):513-518*

## INTRODUCTION

Breast cancer is the most frequent cancer among women, with an estimated of 1.5 million new cases of breast cancer

worldwide in 2002. When both genders are considered together, it is the second most common cancer. Breast cancer is also the leading cause of cancer mortality in women, with 411,000 annual deaths representing 14% of all female cancer deaths. It is also the fifth highest cause of death from cancer overall.<sup>(1)</sup> Several hormone-related factors, such as age at menarche, parity and age at menopause, are associated with breast cancer,<sup>(2)</sup> and high levels of endogenous sex hormones, especially oestrogens, are believed to increase breast cancer risk.<sup>(3-6)</sup> By determining the association between circulating sex steroid hormones and breast cancer risk, we may be able to understand its aetiology better and this may ultimately help us identify high-risk women who would benefit from increased screening or chemoprevention.

## METHODS

A case-control study was conducted involving female patients seen in the surgical department (clinics and wards) of Universiti Kebangsaan Malaysia Medical Centre (UKMMC) from December 2005 to May 2007. Cases recruited were female patients diagnosed with breast cancer, confirmed by histopathological examination in UKMMC. Also recruited were members of the control group, consisting of women in the surgical department (clinics and wards) in UKMMC who were not taking any form of hormonal supplement, including hormone replacement therapy (HRT) and oral contraceptive pills, for the past one year prior to blood sampling, and had not been diagnosed with breast diseases or any other hormone-related tumours for the past one year prior to blood sampling.

Premenopausal women were defined as those women who were still menstruating, not on any HRT and had no previous history of bilateral oophorectomy.<sup>(7)</sup> Postmenopausal patients were defined as those who had attained natural menopause, had a bilateral oophorectomy, or were more than 51 years of age with a history of hysterectomy without oophorectomy.<sup>(8)</sup> Blood was sampled before any treatment was given, i.e. curative/palliative surgery, chemotherapy or radiotherapy. It was taken during the luteal phase, i.e. after the 15th day of the menstrual cycle for the premenopausal women. This blood sample was then sent to the UKMMC biochemistry

Department of Surgery, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latiff, Bandar Tun Razak, Cheras, Kuala Lumpur 56000, Malaysia

Ho CCK, MD, MS, MRCSE Surgeon and Lecturer

Rohaizak M, MBChB, MS, FRCS Professor and Deputy Director

Zulkifli SZ, MD, MMed, PhD Professor and Deputy Director

Department of Pathology

Siti-Aishah MA, MBChB, DCP, MIAC Associate Professor

Nor-Aini U, MD, DCP Associate Professor

Sharifah-Noor-Akmal SH, MBBCh, DCP Professor

Correspondence to: Dr Christopher Ho Chee Kong Tel: (60) 3 9170 2316 Fax: (60) 3 9173 7831 Email: chriskho2002@yahoo.com

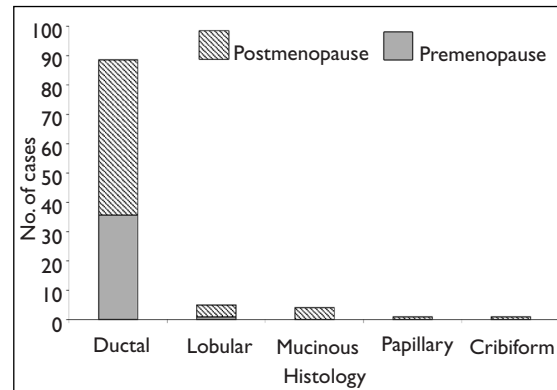
laboratory for analysis. The machine used for oestradiol, progesterone and prolactin analysis was the Architect System, which used immunoassay via Chemiluminescent Microparticle Immunoassay technology with flexible assay protocols, referred to as Chemiflex. For testosterone, the Immulite Analyzer, which is a solid-phase, competitive chemiluminescent enzyme immunoassay, was used.

The data collected was analysed using the Statistical Package for Social Sciences version 11.5 (SPSS Inc, Chicago, IL, USA). The subjects in the study were divided into the premenopausal and postmenopausal groups. Subsequently, these groups were further divided into cases and controls. The race and age distribution were compared. The different serum hormone levels were then grouped into quartiles, with the cut-off points based on the distribution in control subjects. Logistic regression was then used to calculate the adjusted odds ratio (OR) estimates and 95% confidence interval (CI). OR estimates were adjusted for age and race. Due to many cases with a lower than detectable level of oestradiol (< 0.3 nmol/L) among the postmenopausal patients, the material was dichotomised with regard to this hormone. Statistical significance was defined as a  $p$ -value < 0.05.

## RESULTS

A total of 207 subjects were recruited for this study, which was conducted from December 2005 to May 2007. There were 73 premenopausal and 134 postmenopausal subjects. In the premenopausal group, there were 37 cases and 36 controls, while the postmenopausal group had 68 cases and 66 controls. In terms of racial distribution, there were 59 cases and 44 controls among the Malays, 37 cases and 45 controls among the Chinese, and six cases and 11 controls among the Indians. Other racial groups comprised only three cases and two controls. In the premenopausal group, there were 24 cases and 17 controls among the Malays, nine cases and 12 controls among the Chinese, two cases and five controls among the Indians and two cases and two controls in the other racial groups. In the postmenopausal group, there were 35 cases and 27 controls among the Malays, 28 cases and 33 controls among the Chinese, four cases and six controls among the Indians and one case but no control in the other racial groups. In terms of age, the mean for premenopausal breast cancer patients was 43.86 years while for the control group, it was 36.94 years. For the postmenopausal group, the mean age of the breast cancer patients was 59.43 years, while that of the control group was 63.47 years.

The main histology was infiltrating ductal carcinoma, accounting for 89.5% of all breast cancers (Fig. 1). Overall, 67% of breast cancer patients were oestrogen receptor



**Fig. 1** Bar chart shows the distribution of the histological classifications of breast cancer.

positive (ER+) and 76% were progesterone receptor positive (PR+). Among the premenopausal patients, 65.7% were ER+ and 80% were PR+. In postmenopausal patients, 67.7% were ER+ and 73.8% were PR+. Table I shows the OR for breast cancer for the different hormones in premenopausal women, adjusted for age and race. As can be seen, the adjusted OR showed that only testosterone was positively associated with breast cancer. The OR associated with increasing levels of testosterone were 1.00, 1.95 (95% CI 0.42–9.17), 1.11 (95% CI 0.27–4.52) and 1.72 (95% CI 0.40–7.40). However, the OR was not clinically significant ( $p > 0.05$ ). The other hormones, i.e. oestradiol, progesterone and prolactin, were not positively associated with breast cancer. The OR calculated were also not significant ( $p > 0.05$ ).

Table II shows the OR for breast cancer for the different hormones in postmenopausal women, adjusted for age and race. Serum oestradiol, progesterone and testosterone were all positively associated with breast cancer, but only the OR for progesterone and testosterone were clinically significant. The odds of getting breast cancer with testosterone levels in the highest quartile compared to the lowest quartile was 4.23 and this was statistically significant (95% CI 1.52–11.78,  $p = 0.006$ ). The odds of getting breast cancer with higher progesterone levels was 2.35 and this was also significant ( $p = 0.025$ ). For oestradiol, the ORs with increasing levels of serum oestradiol were 1.00, 1.30, 1.57 and 1.48. The odds of getting breast cancer in the highest quartile compared to the lowest quartile for oestradiol was 1.48 (95% CI 0.59–3.69). However, this was not clinically significant ( $p = 0.4$ ). Prolactin was not positively associated with breast cancer.

## DISCUSSION

Multiple epidemiological studies have been carried out to investigate the association between serum sex hormones and premenopausal breast cancer risk, but despite the strength

**Table I. Adjusted odds ratio for breast cancer by concentration of sex-steroid hormones in premenopausal women.**

Hormones	No. of cases	No. of controls	Adjusted OR (95% CI)	p-value
<b>Oestradiol (pmol/L)</b>				
≤ 121.5	8	10	1.00 (referent)	
> 121.5–211.0	11	8	0.40 (0.09–1.79)	0.229
> 211.0–423.5	10	8	0.84 (0.18–3.92)	0.819
> 423.5	8	10	0.51 (0.11–2.48)	0.404
<b>Progesterone (nmol/L)</b>				
≤ 0.3	9	13	1.00 (referent)	
> 0.3–0.9	10	7	0.69 (0.16–2.89)	0.606
> 0.9–14.6	8	8	0.75 (0.16–3.39)	0.704
> 14.6	10	8	0.48 (0.11–2.03)	0.317
<b>Testosterone (ng/ml)</b>				
≤ 0.69	14	9	1.00 (referent)	
> 0.69–1.05	6	7	1.95 (0.42–9.17)	0.396
> 1.05–1.675	9	9	1.11 (0.27–4.52)	0.888
> 1.675	7	11	1.72 (0.40–7.40)	0.468
<b>Prolactin (ug/L)</b>				
≤ 10.595	7	11	1.00 (referent)	
> 10.595–16.565	9	9	0.75 (0.09–2.12)	0.708
> 16.565–30.18	11	7	0.48 (0.11–2.18)	0.344
> 30.18	9	9	0.45 (0.17–3.35)	0.309

of the sex hormone paradigm in breast cancer aetiology, the results have been equivocal.<sup>(4,7)</sup> These inconclusive findings may well reflect the limited number of early onset breast cancer cases that have been studied. Additionally, premenopausal women also experience changes in their serum oestrogen as well as progesterone levels throughout their menstrual cycle and this affected the studies, which did not fix the time during the menstrual cycle for blood sampling. In contrast, studies on postmenopausal women have been more consistent in their results,<sup>(10–13)</sup> because of the absence of the variability of hormone levels with the menstrual cycle.

Experimental studies have shown that oestrogens have a proliferative effect on breast tissue,<sup>(14)</sup> are probably related to increase mitotic activity, and are believed to be a promoting influence rather than an initiating effect.<sup>(15)</sup> The proliferation of cells is essential for carcinogenesis because the risk of errors during deoxyribonucleic acid replication is increased during cell division, which if not corrected, can lead to cancer.<sup>(16,17)</sup> Therefore, oestrogen is believed to increase the risk of breast cancer. A meta-analysis of nine prospective studies on postmenopausal breast cancer showed that the risk for breast cancer increased statistically significantly with increasing concentrations of all sex hormones examined, including oestradiol.<sup>(18)</sup> In 16 case-control studies that compared the serum concentration levels of oestradiol in postmenopausal women, seven of the studies showed a significantly higher mean concentration of oestradiol in breast cancer cases than in controls. Overall,

there was a 24% higher mean concentration of oestradiol in breast cancer cases.<sup>(19)</sup> Studies by Cauley et al,<sup>(20)</sup> Manjer et al<sup>(21)</sup> and Kaaks et al<sup>(22)</sup> showed that the relative risk (RR) and OR for breast cancer in postmenopausal women with the highest concentration of oestradiol was higher compared to women with the lowest concentration. In agreement with previous studies, our study also found a positive association between increasing serum oestradiol levels and the odds of having breast cancer. Unfortunately, it was not statistically significant.

In premenopausal breast cancer cases, the results from ten case-control studies published prior to 1988 were inconsistent.<sup>(4)</sup> Serum oestradiol levels were higher in cases than controls in two of three more recently published case-control studies.<sup>(23–25)</sup> Sturgeon et al showed that mean oestradiol levels in the late follicular stage were lower in cases than controls ( $p \leq 0.05$ ), but there was no significant difference for oestradiol in the late follicular and luteal phases.<sup>(26)</sup> The adjusted OR in our study did not show any positive association between increasing serum oestradiol levels in premenopausal women and the odds of getting breast cancer. Our results concurred with the largest prospective study done recently, conducted in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort on premenopausal women, with 285 invasive breast cancer cases and 555 controls. It showed no association for oestradiol.<sup>(27)</sup> However, in the second large prospective study, conducted within the Nurses' Health Study II (NHSII),<sup>(28)</sup> the results show that follicular, but not luteal,

**Table II. Adjusted odds ratio for breast cancer by concentration of sex-steroid hormones in postmenopausal women.**

Hormones	No. of cases	No. of controls	Adjusted OR (95% CI)	p-value
<b>Oestradiol (pmol/L)</b>				
≤ 37	31	26	1.00 (referent)	
> 37–44	5	5	1.30 (0.33–5.21)	0.711
> 44–72	17	18	1.57 (0.65–3.83)	0.317
> 72	15	17	1.48 (0.59–3.69)	0.400
<b>Progesterone (nmol/L)</b>				
≤ 0.3	48	33	1.00 (referent)	
> 0.3	19	33	2.35 (1.11–4.95)	0.025
<b>Testosterone (ng/ml)</b>				
≤ 0.690	33	26	1.00 (referent)	
> 0.69–0.805	4	1	0.41 (0.04–4.02)	0.446
> 0.805–1.55	19	13	1.00 (0.40–2.49)	0.994
> 1.55	7	25	4.23 (1.52–11.78)	0.006
<b>Prolactin (ug/L)</b>				
≤ 6.65	11	17	1.00 (referent)	
> 6.65–9.66	16	16	0.61 (0.21–1.70)	0.363
> 9.66–16.69	19	17	0.48 (0.17–1.38)	0.173
> 16.69	20	16	0.48 (0.17–1.36)	0.168

total and free oestradiol were significantly associated with breast cancer risk (top to bottom quartile comparison RR = 2.1, 95% CI 1.1–4.1).

Progesterone's role in breast cancer is controversial. It has been hypothesised that its activity of opposing oestrogenic stimulation of the breast decreases breast cancer risk.<sup>(29,30)</sup> On the other hand, some believe that the risk of breast cancer is increased because breast mitotic rates are highest in the luteal (high progesterone) phase of the menstrual cycle.<sup>(30,31)</sup> Murine studies have demonstrated that apoptosis in the mammary gland is inhibited by progesterone,<sup>(32)</sup> and that the progesterone signal contributes to mammary tumour susceptibility.<sup>(7)</sup> For serum progesterone levels in premenopause, the findings of studies have also been conflicting. Two studies showed 9%–12% lower levels,<sup>(8,10)</sup> while a third showed a 97% higher level.<sup>(33)</sup> A number of case-control studies have observed lower levels of serum progesterone in premenopausal cases.<sup>(4,23)</sup> In the large EPIC cohort study, a significant inverse association was observed between progesterone levels and breast cancer risk (top to bottom quartile comparison RR = 0.6, 95% CI 0.4–1.0).<sup>(27)</sup> In the second large study (NHSII), no association was observed between luteal progesterone levels and the risk of breast cancer.<sup>(28)</sup> Our study showed no significant association. In the only study for progesterone levels in postmenopausal breast cancer conducted by Missmer et al, there was no statistically significant association observed (top vs. bottom quartile RR = 0.9, 95% CI 0.6–1.5).<sup>(34)</sup> Our study showed a significant positive association.

Prolactin, which is a polypeptide hormone, is essential for the growth of the mammary gland as well as for lactation.<sup>(35,36)</sup> It has been shown in animals that the administration of exogenous prolactin increases the rate of mammary tumour formation and the suppression of prolactin levels has the opposite effect.<sup>(3,37)</sup> It has also been shown to increase the growth of both normal and malignant breast cells *in vitro*,<sup>(38-40)</sup> although these findings have not been entirely consistent.<sup>(41,42)</sup> In short, prolactin is believed to increase the risk of breast cancer. There has not been any study done on prolactin levels in premenopausal breast cancer. Studies on postmenopausal cases have consistently shown that a higher prolactin level is associated with an increased risk of breast cancer.<sup>(21,43-45)</sup> This is in contrast with the findings of our study. We found an inverse association between serum prolactin and both pre- and postmenopausal breast cancer. This inverse association was not significant. This can be attributed to the fact that circulating prolactin has a strong circadian variation which we did not account for. It increases substantially with a noon-time meal and postmenopausally, tends to fluctuate more over time (within a woman) than most other sex steroid hormones.<sup>(44)</sup> Therefore, we should have closely matched the cases and controls on both time of day of blood draw and fasting status. In addition, certain medications like reserpine, haldol, cimetidine and phenothiazine, are known to increase plasma prolactin levels, whereas drugs like levodopa, decrease plasma prolactin levels.<sup>(46)</sup> This had not been taken into account in this study.

Testosterone is believed to indirectly affect breast

tissue by promoting tumour development.<sup>(3)</sup> By binding competitively to sex-hormone binding globulin (SHBG) due to its stronger affinity to this protein, testosterone also affects the fraction of bioavailable oestradiol.<sup>(21)</sup> In this way, it increases the amount of free oestradiol, therefore indirectly increasing the risk of breast cancer. Studies on testosterone in premenopausal cases have been inconsistent.<sup>(8,26,33)</sup> Some studies showed no association, while others reported positive associations.<sup>(47-49)</sup> The large EPIC cohort<sup>(27)</sup> showed significant positive associations between circulating levels of testosterone and the risk of breast cancer. In the NHSII study,<sup>(28)</sup> modest but not statistically significant positive associations were observed for testosterone. In our study, the adjusted OR showed a positive association, where increasing levels of serum testosterone were found to be at higher odds of having premenopausal breast cancer. However, this was not statistically significant.

In contrast, postmenopausal breast cancer studies have consistently shown that higher levels of testosterone are associated with breast cancer.<sup>(11,34,39)</sup> The pooled analysis of nine prospective studies concluded that breast cancer risk increased with increasing testosterone levels: the RRs (95% CI) for increasing quintile (all relative to the lowest quintile) were 1.3, 1.6, 1.6 and 2.2 (1.6–3.1).<sup>(48)</sup> The results of our study were also consistent with these findings. The limitations of our study include its cross-sectional design and the fact that the results are based on hormone measurements in a blood sample collected at a single time point. These single measurements provide an imperfect estimate of an individual woman's long-term serum hormone levels. Secondly, hormone levels in premenopausal women, in particular oestradiol and progesterone, fluctuate with the menstrual cycle, and it was difficult to get the premenopausal women to comply with the regulation of getting their blood assayed at a certain day of their menstrual cycle. Therefore, blood-taking for premenopausal women was not standardised. Thirdly, the sample size for a study of this proportion was not adequate due to financial and time constraints.

In summary, there was no statistically significant association between serum sex hormones and breast cancer in premenopausal patients. In postmenopausal women, serum progesterone and testosterone levels were significantly associated positively with the odds of having breast cancer. Our study therefore suggests that serum sex hormone levels do not play a role in predicting the odds of having breast cancer in premenopausal women. In postmenopausal women, high levels of progesterone and testosterone hormones predict a higher odds of having breast cancer. Hence, our study suggests that

postmenopausal women with high levels of progesterone or testosterone hormone should be screened for breast cancer.

## ACKNOWLEDGEMENTS

Financial support for this study was provided by IRPA fund no. 09-02-02-009 BTK/ER/37/2. Approval for this study was granted by the Research and Ethical Committee of UKM Medical Centre.

## REFERENCES

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; 55:74-108.
2. Hsieh C, Trichopoulos D, Katsouyanni K, Yuasa S. Age at menarche, age at menopause, height and obesity as risk factors of breast cancer: associations and interactions in an international case-control study. *Int J Cancer* 1990; 46:796-800.
3. Bernstein L, Ross K. Endogenous hormones and breast cancer risk. *Epidemiol Rev* 1993; 15:48-65.
4. Key TJ, Pike MC. The role of oestrogens and progestogens in the epidemiology and prevention of breast cancer. *Eur J Cancer Clin Oncol* 1988; 24:29-43.
5. Pike MC, Spicer DV, Dahmouh L, Press MF. Estrogens, progestogens, normal breast cell proliferation, and breast cancer risk. *Epidemiol Rev* 1993; 15:17-35.
6. Helzlsouer KJ, Alberg A, Bush TL, et al. A prospective study of endogenous hormones and breast cancer. *Cancer Detect Prev* 1994; 18:79-85.
7. Haslam SZ, Osuch JR, Raafat AM, Hofseth LJ. Postmenopausal hormone replacement therapy: effects on normal mammary gland in humans and in a mouse postmenopausal model. *J Mammary Gland Biol Neoplasia* 2002; 7:93-105.
8. Wysowski DK, Comstock GW, Helsing KJ, Lau HL. Sex hormone levels in serum in relation to the development of breast cancer. *Am J Epidemiol* 1987; 125:791-9.
9. Key TJ, Verkasalo PK. Endogenous hormones and the aetiology of breast cancer. *Breast Cancer Res* 1999; 1:18-21.
10. Thomas HV, Key TJ, Allen DS, et al. A prospective study of endogenous serum hormone concentrations and breast cancer risk in premenopausal women on the island of Guernsey. *Br J Cancer* 1997; 75:1075-9.
11. Dorgan JF, Longcope C, Stephenson HE Jr, et al. Relation of prediagnostic serum estrogen and androgen levels to breast cancer risk. *Cancer Epidemiol Biomarkers Prev* 1996; 5:533-9.
12. Hankinson SE, Willett WC, Manson JE, et al. Plasma sex steroid hormone levels and risk of breast cancer in postmenopausal women. *J Natl Cancer Inst* 1998; 90:1292-9.
13. Thomas HV, Reeves GK, Key TJ. Endogenous estrogen and postmenopausal breast cancer: a quantitative review. *Cancer Causes Control* 1997; 8:922-8.
14. David BT. Do hormones cause breast cancer? *Cancer* 1984; 53 (3 Suppl):595-604.
15. Telang NT, Katdare M, Bradlow HL, Osborne MP. Estradiol metabolism: an endocrine biomarker for modulation of human mammary carcinogenesis. *Environ Health Perspect* 1997; 105 Suppl 3:559-64.
16. Osborne MP, Bradlow HL, Wong GY, Telang NT. Upregulation of estradiol C16 alpha-hydroxylation in human breast tissue: a potential biomarker of breast cancer risk. *J Natl Cancer Inst* 1993; 85:1917-20.
17. Henderson BE, Pike MC, Bernstein L, Ross RK. Breast cancer. In: Schottenfeld D, Fraumeni JF, eds. *Cancer Epidemiology and Prevention*. 2nd ed. New York: Oxford University Press, 1996.



18. Key T, Appleby P, Barnes I, Reeves G, Endogenous Hormones and Breast Cancer Collaborative Group. Endogenous hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. *J Natl Cancer Inst* 2002; 94:606-16.
19. Thomas HV, Reeves GK, Key TJ. Endogenous estrogen and postmenopausal breast cancer : a quantitative review. *Cancer Causes Control* 1997; 8:922-8.
20. Cauley JA, Lucas FL, Kuller LH, et al. Elevated serum estradiol and testosterone concentrations are associated with a high risk for breast cancer. *Ann Intern Med* 1999; 130(4 Pt 1):270-7.
21. Manjer J, Johansson R, Berglund G, et al. Postmenopausal breast cancer risk in relation to sex steroid hormones, prolactin and SHBG (Sweden). *Cancer Causes Control* 2003; 14:599-607.
22. Kaaks R, Rinaldi S, Key TJ, et al. Postmenopausal serum androgens, oestrogens and breast cancer risk: the European prospective investigation into cancer and nutrition. *Endocr Relat Cancer* 2005; 12:1071-82.
23. Bernstein L, Yuan JM, Ross RK, et al. Serum hormone levels in pre-menopausal Chinese women in Shanghai and white women in Los Angeles :results from two breast cancer case-control studies. *Cancer Causes Control* 1990; 1:51-8.
24. Zaridze D, Kushlinskii N, Moore JW, et al. Endogenous plasma sex hormones in pre- and postmenopausal women with breast cancer: results from a case control study in Moscow. *Eur J Cancer Prev* 1992; 1:225-30.
25. Malarkey WB, Schroeder LL, Stevens VC, James AG, Lanese RR. Twenty-four-hour preoperative endocrine profiles in women with benign and malignant breast disease. *Cancer Res* 1977; 37:4655-9.
26. Sturgeon SR, Potischman N, Malone KE, et al. Serum levels of sex hormones and breast cancer risk in premenopausal women: a case-control study (USA). *Cancer Causes Control* 2004; 15:45-53.
27. Kaaks R, Berrino F, Key T, et al. Serum sex steroids in premenopausal women and breast cancer risk within the European Prospective Investigation into Cancer and Nutrition (EPIC). *J Natl Cancer Inst* 2005; 97:755-65.
28. Eliassen AH, Missmer SA, Tworoger SS, et al. Endogenous steroid hormone concentrations and risk of breast cancer among premenopausal women. *J Natl Cancer Inst* 2006; 98:1406-15.
29. Kelsey JL. A review of the epidemiology of human breast cancer. *Epidemiol Rev* 1979; 1:74-109.
30. Foidart JM, Colin C, Denoo X, et al. Estradiol and progesterone regulate the proliferation of human breast epithelial cells. *Fertil Steril* 1998; 69:963-9.
31. Harris JR, Lippman ME, Veronesi U, Willett W. Breast cancer (3). *N Engl J Med* 1992; 327:473-80.
32. Haslam SZ, Counterman LJ. Mammary stroma modulates hormonal responsiveness of mammary epithelium in vivo in the mouse. *Endocrinology* 1991; 129:2017-23.
33. Helzlsouer KJ, Alberg AJ, Bush TL, et al. A prospective study of endogenous hormones and breast cancer. *Cancer Detect Prev* 1994; 18:79-85.
34. Missmer SA, Eliassen AH, Barbieri RL, Hankinson SE. Endogenous estrogen, androgen, and progesterone concentrations and breast cancer risk among postmenopausal women. *J Natl Cancer Inst* 2004; 96:1856-65.
35. Vonderhaar BK. Prolactin: transport, function and receptors in mammary gland development and differentiation. In: Neville MC, Daniel CW, eds. *The Mammary Gland: Development, Regulation and Function*. New York: Plenum Press, 1987; 383-438.
36. Meites J. Biological functions of prolactin in mammals. In: Hoshino K, ed. *Prolactin Gene Family and Its Receptors. Molecular Biology to Clinical Problems. Proceedings of the Fifth International Congress on Prolactin*. Kyoto, Japan: Elsevier, 1998: 123-30.
37. Welsch CW, Nagasawa H. Prolactin and murine mammary tumorigenesis: a review. *Cancer Res* 1977; 37:951-63.
38. Peyrat JP, Djiane J, Bonnetterre J, et al. Stimulation of DNA synthesis by prolactin in human breast tumor explants. Relation to prolactin receptors. *Anticancer Res* 1984; 4:257-61.
39. Malarkey WB, Kennedy M, Allred LE, Milo G. Physiological concentrations of prolactin can promote the growth of human breast tumor cells in culture. *J Clin Endocrinol Metab* 1983; 56:673-7.
40. Simon WE, Albrecht M, Trams G, Dietel M, Hölzel F. In vitro growth promotion of human mammary carcinoma cells by steroid hormones, tamoxifen, and prolactin. *J Natl Cancer Inst* 1984; 73:313-21.
41. Calaf G, Garrido F, Moyano C, Rodríguez R. Influence of hormones on DNA synthesis of breast tumors in culture. *Breast Cancer Res Treat* 1986; 8:223-32.
42. Beeby DI, Easty GC, Gazet JC, Grigor K, Neville AM. An assessment of the effects of hormones on short term organ cultures of human breast carcinomata. *Br J Cancer* 1975; 31:317-28.
43. Wang DY, De Stavola BL, Bulbrook RD, et al. Relationship of blood prolactin levels and the risk of subsequent breast cancer. *Int J Epidemiol* 1992; 21:214-21.
44. Hankinson SE, Willett WC, Michaud DS, et al. Plasma prolactin levels and subsequent risk of breast cancer in postmenopausal women. *J Natl Cancer Inst* 1999; 91:629-34.
45. Kabuto M, Akiba S, Stevens RG, Neriishi K, Land CE. A prospective study of estradiol and breast cancer in Japanese women. *Cancer Epidemiol Biomarkers Prev* 2000; 9:575-9.
46. Yen SS, Jaffe RB, eds. *Reproductive Endocrinology*. 3rd ed. Philadelphia: Saunders, 1991.
47. Secreto G, Toniolo P, Berrino F, et al. Increased androgenic activity and breast cancer risk in premenopausal women. *Cancer Res* 1984; 44(12 Pt 1): 5902-5.
48. Secreto G, Toniolo P, Pisani P, et al. Androgens and breast cancer in premenopausal women. *Cancer Res* 1989; 49:471-6.
49. Secreto G, Recchione C, Fariselli G, Di Pietro S. High testosterone and low progesterone circulating levels in premenopausal patients with hyperplasia and cancer of the breast. *Cancer Res* 1984; 44:841-4.