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Reproductive factors, exogenous hormone use and risk of hepatocellular carcinoma among US women: results from the Liver Cancer Pooling Project

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Background: Hepatocellular carcinoma (HCC) occurs less commonly among women than men in almost all regions of the world. The disparity in risk is particularly notable prior to menopause suggesting that hormonal exposures during reproductive life may be protective. Exogenous oestrogenic exposures such as oral contraceptives (OCs), however, have been reported to increase risk, suggesting that estrogens may be hepatocarcinogenic. To examine the effects of reproductive factors and exogenous hormones on risk, we conducted a prospective analysis among a large group of US women.

Methods: In the Liver Cancer Pooling Project, a consortium of US-based cohort studies, data from 799 500 women in 11 cohorts were pooled and harmonised. Cox proportional hazards regression models were used to generate hazard ratios (HRs) and 95% confidence intervals (CIs) for the associations of reproductive factors and exogenous hormones with HCC ($n = 248$).

Results: Bilateral oophorectomy was associated with a significantly increased risk of HCC (HR = 2.67, 95% CI = 1.22–5.85), which did not appear to be related to a shorter duration of exposure to endogenous hormones or to menopausal hormone therapy use. There was no association between OC use and HCC (HR = 1.12, 95% CI = 0.82–1.55). Nor were there associations with parity, age at first birth, age at natural menopause, or duration of fertility.

Conclusions: The current study suggests that bilateral oophorectomy increases the risk of HCC but the explanation for the association is unclear. There was no association between OC use and HCC risk. Examination of endogenous hormone levels in relation to HCC may help to clarify the findings of the current study.

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Hepatocellular carcinoma (HCC), the dominant histologic type of primary liver cancer, occurs two to three times less frequently among women than men (McGlynn and London, 2011). Women also have better survival rates and lower recurrence rates after HCC treatment than do men (Ng *et al*, 1997; Fukuda *et al*, 2007). The explanation for this gender disparity is not clear. Although some major risk factors, such as infection with hepatitis B virus (HBV), hepatitis C virus (HCV), excessive alcohol consumption, and cigarette smoking are more common among men, these factors do not explain, completely, the gender differences in incidence or outcome (McGlynn and London, 2011). Such differences are not as pronounced among men and postmenopausal women (Shimizu and Ito, 2007), suggesting that hormonal factors during reproductive life may be associated with reduced risk.

Early animal experiments that examined the effects of hormones on chemically induced liver tumours suggested that estrogens promoted hepatocarcinogenesis (Yager and Yager, 1980; Cameron *et al*, 1981; Wanless and Medline, 1982). In contrast, other experiments have reported a tumor-enhancing effect of ovariectomy on liver cancers (Vesselinovitch *et al*, 1980; Nakatani *et al*, 2001). In addition, rodent experiments have demonstrated the ability of estrogens to protect against diethylnitrosamine-induced liver cancer due to their ability to inhibit the production of interleukin-6 (IL-6), a multifunctional cytokine (Naugler *et al*, 2007). Whether a similar phenomenon occurs in human liver cancer is not clear.

Findings from human observational studies in regard to hormonal exposures have been contradictory. For example, some studies have suggested higher parity increases risk (Plesko *et al*, 1985; La Vecchia *et al*, 1992; Stanford and Thomas, 1992), while others have suggested that higher parity decreases risk (Yu *et al*, 2003; Fwu *et al*, 2009; Kanazir *et al*, 2010; Wu *et al*, 2011). Similarly, an association between oral contraceptive (OC) use and liver cancer has remained uncertain. Although the International Agency for Research on Cancer (IARC) concluded in 1999 that there was sufficient evidence that OCs increased risk of HCC in the absence of viral infections (1999), a meta-analysis of the same studies later concluded that the evidence for a link was uncertain (Maheshwari *et al*, 2007).

Very few prior studies of reproductive factors and HCC have been conducted in the US (Yu *et al*, 1991; Hsing *et al*, 1992a, b), and all prior US studies have included fewer than 75 cases. Thus, we conducted an examination of the relationship of reproductive factors and exogenous hormone use with primary liver cancer and HCC in a large pooled study of US women.

MATERIALS AND METHODS

Study population. All US-based cohort studies that are members of the National Cancer Institute (NCI) Cohort Consortium (http://epi.grants.cancer.gov/Consortia/cohort.html) were invited to participate in the Liver Cancer Pooling Project. For the current analysis, which is restricted to female participants, 11 cohort studies elected to participate: NIH-AARP Diet and Health Study, Agricultural Health Study, United States Radiologic Technologists Study, Breast Cancer Detection Demonstration Project Follow-Up Study, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial, Women's Health Study, New York University Women's Health Study, Cancer Prevention Study II, Iowa Women's Health Study, Black Women's Health Study, and Women's Health Initiative (Supplementary Table 1). All studies contributed de-identified data from the entire cohort following data sharing agreements approved by the NCI and each cohort's academic institution. Studies with stored serum samples also provided samples from a subset of the population to be used for HBV and HCV testing. For the serum subset, female controls were matched to cases on age at a 2 : 1 ratio.

To be considered an HCC case, cohort members had to have developed HCC documented by a cancer registry report or a medical record report. HCCs were identified by ICD-10 topography code C22 (www.cdc.gov/nchas/icd/icd10.htm) and ICD-O-3 morphology code 8170–8175 (www.who.int/classifications/icd/adaptations/oncology/en/). All cases had to be diagnosed after the cohort participant completed and returned her initial questionnaire to the parent cohort.

Among the 11 participating US-based cohorts, 837 217 women were participants. For the current analysis, 3 women were excluded due to a prior liver cancer diagnosis, and 10 459 women were excluded due to having zero follow-up time. In addition, 27 255 women from the Women's Health Initiative were excluded because they were randomised to the menopausal hormone therapy (MHT) study arm. After exclusions, 799 500 female members of the 11 cohorts remained for the current study. During the course of follow-up, 248 women developed HCC.

Exposures. Reproductive factors of primary interest to the analysis included age at menarche, ever giving birth, number of children, age at first birth, age at natural menopause, bilateral oophorectomy, hysterectomy, ever use of OCs, duration of use of OCs, ever use of MHT, recency of MHT use (never, current, and former), duration of MHT use, type of MHT use (none, estrogen-only MHT, and estrogen–progesterone combination MHT), and MHT route of administration (none, oral, and non-oral). Duration of OC use was categorised in two ways as some prior studies had hypothesised that risk of HCC is not increased prior to 5 years of use (IARC, 1999). Thus, OC use was both dichotomised (<5 and $\geqslant 5$ years) and was examined in shorter intervals of years (<1, 1 to <3, 3 to <6, 6 to <8, and $\geqslant 8$). For MHT use, only some cohorts had information on duration, formulation, and route of administration, thus analyses of those variables were based on fewer women than analyses of ever use and timing of use. If a woman indicated that she had undergone both a bilateral oophorectomy and a hysterectomy, she was only included in the oophorectomy group. Women were included in the hysterectomy group only if they did not report having had an oophorectomy.

Laboratory methods. Serum samples were analysed for markers of HBV and HCV infection. For HBV, hepatitis B surface antigen (HBsAg) was detected using the Bio-Rad GS HBsAg 3.0 enzyme immunoassay (Bio-Rad Laboratories, Redmond, WA, USA) and antibody to hepatitis B core antigen (anti-HBc) was detected using the Ortho HBc ELISA test system (Ortho-Clinical Diagnostics, Inc. Raritan, NJ, USA). For HCV, antibody to HCV (anti-HCV) was detected using the Ortho HCV Version 3.0 ELISA test system (Ortho-Clinical Diagnostics, Inc.) and positive results were confirmed using the Chiron RIBA HCV 3.0 SIA (Ortho-Clinical Diagnostics, Inc.). All analyses were conducted in the Protein Expression Laboratory at the Frederick National Laboratory for Cancer Research, Frederick, MD, USA, under the direction of Dr Rachel Bagni.

Statistical analysis. Cox proportional hazards regression models, with follow-up time as the time metric, were used to determine hazard ratios (HRs), as approximations of relative risks, and 95% confidence intervals (CIs) for the associations between reproductive factors and HCC. Initially, parsimonious models were employed that adjusted only for age (continuous) and parent cohort study. Subsequently, more inclusive models were examined that also adjusted for: alcohol consumption (grams per day; non-drinkers, $\leqslant 1.08$, >1.08–3.58, >3.58–13.54, and >13.54), BMI (<25, 25–29 and $\geqslant 30\,\mathrm{kg\,m^{-2}}$), diabetes (no or yes), race (white or other), smoking status (never, former, and current), education (some high school or less/high school degree or GED/some college

or vocational training/college degree/post college education). The fully adjusted model that assessed reproductive factors was also adjusted for menopausal status (premenopausal or postmenopausal), while the models examining menopausal factors (age at menopause and MHT use) were restricted to postmenopausal women. In addition to the overall analyses, sensitivity analyses which excluded the first year, and the first 2 years after baseline of follow-up, were conducted, as was an analysis which excluded virally infected cases. The proportional hazard assumption was satisfied for analyses using Cox proportional hazards modelling.

Statistical significance in all analyses was set at $P<0.05$ based on two-sided tests. All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Characteristics of the women in the Liver Cancer Pooling Project are displayed in Table 1. A majority of the participants were white (84.5%), married or living as married (63.5%), postmenopausal (86.7%), and were non-smokers (53.5%).

Table 2 displays the relationship between reproductive factors and HCC among all of the participants. No associations were evident with age at menarche, parity (either ever having children or number of children), or age at first birth. Similarly, there was no association with use of OCs (HR = 1.12, 95% CI = 0.82–1.55). An examination of duration of OC use also found no association with HCC, whether duration was categorised as <5 years *vs* $\geqslant 5$ years, or was broken down more finely.

Table 3 displays the relationship between menopausal factors and MHT and HCC. Although there was no association with age at menopause, there was a significantly increased risk of HCC associated with bilateral oophorectomy (HR = 2.01; 95% CI = 1.12–3.61). There was no association with total fertile duration, however. The analysis of MHT use found a modestly increased risk of HCC (HR = 1.35, 95% CI = 1.01–1.81), which was more evident for use of estrogen-only MHT (HR = 1.57, 95% CI = 1.05–2.35). Dividing the ever users into current or former users did not alter the results, nor did examining the duration of use. Analysis by route of administration revealed a significantly increased risk only in association with non-oral MHT, however, the result was based on only five cases.

As women who have bilateral oophorectomies prior to menopause are likely to use MHT, particularly estrogen-only MHT, analyses were run which adjusted each variable for the other. As shown in Table 4, when the analysis of bilateral oophorectomy was adjusted for MHT use, bilateral oophorectomy remained significantly associated with risk of HCC (HR = 1.92, 95% CI = 1.04–3.53). However, when the analysis of MHT use was adjusted for bilateral oophorectomy, neither MHT use (HR = 1.15, 95% CI = 0.81–1.63 for MHT use and HR = 1.09, 95% CI = 0.63–1.88 for estrogen-only MHT use) remained significantly associated with HCC.

The examination of HBV and HCV status among a subset of the participants found, as anticipated, that HBV and HCV infections were more common among the cases than the controls. Among the 82 HCC cases tested, 31.7% ($n=26$) were positive for anti-HCV and 3.7% ($n=3$) were positive for HBsAg, compared to the 177 controls where 2.3% ($n=4$) were anti-HCV positive and 0.6% ($n=1$) were HBsAg positive. The viral results could not be incorporated into the larger analyses, as the results were only available for a small proportion of the cases and an even smaller proportion of the controls. Sensitivity analyses were conducted, however, that dropped anti-HCV(+) and HBsAg(+) cases. The results of these analyses did not differ from the analyses that included all cases (data not shown). Similarly, the analyses that dropped cases that developed in the first year of follow-up, or in the first 2 years, had very similar results as the analyses that included all follow-up time (data not shown).

Table 1. Characteristics of women in the Liver Cancer Pooling Project

	Total cohort ($N=799\,050$)	
	N	%
Person-years	8 941 402	
Age at entry (years)		
<50	127 804	16.0
50–59	257 409	32.2
60–69	334 570	41.9
⩾70	79 267	9.9
Race		
White	668 163	84.5
Black	93 314	11.8
Asian/Pacific Islander	10 965	1.4
American Indian/Alaska Native	2029	0.3
Other	15 754	2.0
Missing	8825	—
Body mass index (kg m^{-2})		
<25	353 321	45.7
25–29.9	250 330	32.3
⩾30	169 936	22.0
Missing	25 463	—
Education		
Some high school or less	45 498	5.9
High school	184 095	23.9
Some college/vocational	285 764	37.1
College degree	128 277	16.7
Post college education	126 506	16.4
Missing	28 910	—
Marital status		
Married/living as married	494 230	63.5
Not married/not living as married	283 714	36.5
Missing	21 106	—
Menopausal status		
Premenopausal	105 238	13.3
Postmenopausal	683 399	86.7
Missing	10 413	—
Diabetes		
No	741 149	94.4
Yes	44 020	5.6
Missing	13 881	—
Alcohol (grams per day)		
Non-drinker	248 628	33.0
⩽1.08	180 449	24.0
>1.08–3.58	121 278	16.1
>3.58–13.54	118 449	15.7
>13.54	84 515	11.2
Missing	45 731	—
Cigarette smoking status		
Non-smoker	418 382	53.5
Former smoker	272 081	34.8
Current smoker	91 909	11.7
Missing	16 678	—

DISCUSSION

In the current pooled analysis of US-based studies, bilateral oophorectomy was associated with a significantly increased risk of HCC. Although MHT use, in particular estrogen-only MHT use, appeared to be associated with risk, the association was attenuated and no longer significant once adjustment was made for bilateral

Table 2. Association between reproductive factors and hepatocellular carcinoma among women, the Liver Cancer Pooling Project

	HCC (*N* = 203)	Non-cases (*N* = 677 183)	HR[a]	95% CI
Age at menarche (years)				
<12	69	197 575	1.00	Referent
12–13	104	343 392	0.95	0.69, 1.31
14+	27	125 507	0.64	0.40, 1.03
Missing	3	10 709	—	—
P-trend				0.09
Ever had children				
No	27	100 196	1.00	Referent
Yes	172	564 516	0.85	0.57, 1.29
Missing	4	12 471	—	—
Number of children				
0	27	100 256	1.00	Referent
1	17	78 149	0.75	0.41, 1.38
2	39	176 882	0.72	0.44, 1.19
3–4	80	234 041	0.92	0.59, 1.43
5+	35	74 173	0.97	0.58, 1.62
Missing	5	13 682	—	—
P-trend				0.46
Age at first birth (years; parous women)				
<21	38	105 631	1.00	Referent
21–24	82	247 902	1.02	0.68, 1.52
25–28	31	130 762	0.75	0.45, 1.25
⩾29	12	55 266	0.72	0.37, 1.43
Missing	40	137 622	—	—
P-trend				0.11
Oral contraceptive use				
No	132	351 467	1.00	Referent
Yes	69	319 898	1.12	0.82, 1.55
Missing	2	5818	—	—
Duration of oral contraceptive use (years)				
None	132	351 467	1.00	Referent
<1	9	45 398	0.95	0.47, 1.91
1 to <3	19	89 858	1.00	0.61, 1.64
3 to <6	7	46 182	1.03	0.47, 2.25
6 to <8	15	61 242	1.27	0.73, 2.22
8+	17	72 756	1.27	0.75, 2.15
Missing	4	10 280	—	—
P-trend				0.40
<5	34	171 742	1.01	0.68, 1.51
⩾5	33	143 694	1.22	0.82, 1.83
Missing	4	10 280	—	—

Abbreviations: BMI = body mass index; CI = confidence interval; HCC = hepatocellular carcinoma; HR = hazard ratio.
[a]Adjusted for age, alcohol, BMI, diabetes, race, smoking, parent cohort study, menopausal status, and education.

Table 3. Associations between reproductive factors and hepatocellular carcinoma among postmenopausal women, the Liver Cancer Pooling Project

	HCC (*N* = 200)	Non-cases (*N* = 586 271)	HR[a]	95% CI
Age at menopause				
Natural menopause				
<45	9	34 502	0.79	0.39, 1.61
45–49	29	83 693	1.14	0.72, 1.81
50–54	47	151 578	1.00	Referent
⩾55	7	34 443	0.56	0.25, 1.24
P-trend				0.77
Surgical menopause				
Bilateral oophorectomy	22	50 590	2.01	1.12, 3.61
Hysterectomy	43	99 364	1.32	0.83, 2.10
Missing	43	132 101	—	—
Fertile duration[b,c]				
All women	111	327 583	0.98	0.95, 1.02
Natural menopause	91	301 280	1.00	0.96, 1.04
Bilateral oophorectomy	18	23 066	0.98	0.92, 1.04
Hysterectomy	2	3237	—	—
MHT				
Ever used MHT				
Never	86	246 283	1.00	Referent
Ever use	112	335 084	1.35	1.01, 1.81
Missing	2	4904	—	—
Timing of use				
Never	83	228 170	1.00	Referent
Former	37	74 793	1.41	0.95, 2.09
Current	68	229 139	1.28	0.92, 1.79
Missing	12	54 169	—	—
Duration of use[c] (years)				
None	43	136 437	1.00	Referent
<5	20	60 255	1.57	0.91, 2.69
5–9	5	39 801	0.70	0.27, 1.77
10+	21	68 777	1.46	0.86, 2.50
Missing	100	266 779	—	—
P-trend				0.70
MHT formulation[c]				
None	45	152 060	1.00	Referent
Estrogen only	51	124 604	1.57	1.05, 2.35
Combination	17	99 557	0.85	0.48, 1.51
Unknown	6	15 827	2.11	0.88, 5.05
Missing	70	179 362	—	—
Route of administration[c]				
None	32	87 413	1.00	Referent
Oral	38	117 653	1.33	0.82, 2.15
Non-oral	5	8412	2.67	1.02, 7.03
Missing	114	357 932	—	—

Abbreviations: BMI = body mass index; CI = confidence interval; HCC = hepatocellular carcinoma; HR = hazard ratio; MHT = menopausal hormone therapy.
[a]Adjusted for age, alcohol, BMI, diabetes, race, smoking, parent cohort study, and education.
[b]Analysis examines per 1 year increase in fertile duration.
[c]Questions were only ascertained among a subset of cohort participants.

oophorectomy. Oral contraceptive use was not associated with risk. The other reproductive variables were also not associated with risk of HCC.

The results on the current study in regard to bilateral oophorectomy agree with the sole prior study to examine a relationship between oophorectomy and HCC (Yu *et al*, 2003). That result, from a high-rate country, and the current result, are also consistent with the results of animal studies, which have reported an increased risk of liver cancer and accelerated growth of liver tumours after ovariectomy (Vesselinovitch *et al*, 1980; Goldfarb and Pugh, 1990; Nakatani *et al*, 2001). The association with oophorectomy, however, appears somewhat inconsistent with the lack of association with age at natural menopause. The current study also found no association between total duration of fertility and risk, suggesting that the increased risk with bilateral oophorectomy might be related to factors other than a decrease in estrogen levels. For example, oophorectomy has been shown to alter lipid levels among humans (Lobo, 2007) and to increase hepatic androgen receptors among rodents (Tejura *et al*, 1989). In addition, several studies have reported increased mortality risk after oophorectomy, though no study has specifically examined HCC (Gierach *et al*, 2013; Parker *et al*, 2013).

In prior studies, the reproductive factor most frequently examined for a relationship with liver cancer has been parity (Miller *et al*, 1980; Plesko *et al*, 1985; La Vecchia *et al*, 1992; Stanford and Thomas, 1992; Tzonou *et al*, 1992; Hsing *et al*, 1992b; Lambe *et al*, 1993; Kvale *et al*, 1994; Mucci *et al*, 2001; Yu *et al*, 2003;

Table 4. Associations of oophorectomy and MHT with hepatocellular carcinoma after adjustment of each variable for the other, the Liver Cancer Pooling Project

	HR[a]	95% CI
Age at menopause[b]		
Natural menopause		
<45	0.70	0.33, 1.49
45–49	1.14	0.72, 1.81
50–54	1.00	Referent
⩾55	0.56	0.25, 1.24
Surgical menopause		
Bilateral oophorectomy	1.92	1.04, 3.53
Hysterectomy	1.25	0.78, 2.01
Missing	—	—
MHT[c]		
Ever used MHT		
Never	1.00	Referent
Ever use	1.15	0.81, 1.63
Missing	—	—
MHT formulation		
None	1.00	Referent
Estrogen only	1.09	0.63, 1.88
Combination	0.77	0.39, 1.54
Unknown	2.13	0.87, 5.22
Missing	—	—

Abbreviations: BMI = body mass index; CI = confidence interval; HR = hazard ratio; MHT = menopausal hormone therapy.
[a]Adjusted for age, alcohol, BMI, diabetes, race, smoking, parent cohort study, and education.
[b]Also adjusted for MHT.
[c]Also adjusted for age at menopause.

Fwu *et al*, 2009; Kanazir *et al*, 2010; Wu *et al*, 2011). Studies before 1993 reported either increased risk of HCC with increasing parity (Plesko *et al*, 1985; La Vecchia *et al*, 1992; Stanford and Thomas, 1992) or null associations (Miller *et al*, 1980; Tzonou *et al*, 1992; Hsing *et al*, 1992b). Subsequent studies, however, reported either decreased risks with increasing parity (Yu *et al*, 2003; Fwu *et al*, 2009; Kanazir *et al*, 2010; Wu *et al*, 2011) or null associations (Lambe *et al*, 1993; Kvale *et al*, 1994; Mucci *et al*, 2001). As several of the earlier studies were from regions where HBV is the dominant risk factor (La Vecchia *et al*, 1992; Stanford and Thomas, 1992), it was suggested that parity might only increase risk among HBV(+) women (Tzonou *et al*, 1992). This hypothesis, however, has not been supported by more recent, larger, studies from Taiwan, where HBV is the dominant risk factor. Three studies from Taiwan have reported decreased risks with increasing parity (Yu *et al*, 2003; Fwu *et al*, 2009; Wu *et al*, 2011) and one of the studies (Yu *et al*, 2003) found no difference in the parity–HCC relationship between HBV(+) and HBV(−) women. The reasons for the inconsistency in results between earlier and later studies may be due to the relatively small (<80) number of cases in the earlier studies (Miller *et al*, 1980; La Vecchia *et al*, 1992; Stanford and Thomas, 1992; Tzonou *et al*, 1992; Hsing *et al*, 1992b) and the examination of liver cancer, rather than HCC, as the main outcome. In addition, some studies were unable to adjust for other risk factors (Stanford and Thomas, 1992) or retrieved parity information solely from death certificates (Plesko *et al*, 1985). The current finding of a null association with parity is consistent with the results of the only prior US study (Hsing *et al*, 1992b), and with the results of studies from other low-rate HCC countries such as Canada, Sweden, and Norway (Miller *et al*, 1980; Lambe *et al*, 1993; Kvale *et al*, 1994). Why parity would be inversely associated with HCC in high-rate countries, but not in low-rate is unclear, but may be related to other, undetermined factors.

Age at first birth has been examined in six prior studies, of which five found no evidence of a relationship with liver cancer (Miller *et al*, 1980; La Vecchia *et al*, 1992; Stanford and Thomas, 1992; Tzonou *et al*, 1992; Lambe *et al*, 1993). The results of the current study agree with these findings. One prior study from a high-rate region, however (Wu *et al*, 2011), reported that older age at first birth increased risk. As that study also found that higher parity decreased risk, the age-at-first-birth finding perhaps was not surprising. In that study, the ages at first birth were higher than in the current study so it is possible that older ages at first birth (>30 years) could confer increased risk. Too few women in the current study gave birth for the first time at these ages to permit examination of that hypothesis.

Age at menarche has been reported to have no association with liver cancer risk by three studies (La Vecchia *et al*, 1992; Tzonou *et al*, 1992; Kanazir *et al*, 2010), while two studies reported that later age at menarche decreased risk (Mucci *et al*, 2001; Yu *et al*, 2003). Although the current study found no significant association with HCC, the risk (HR = 0.64) was lowest among women with menarche at ages 14+ years. It is thus conceivable that if there were greater numbers of women with older ages at menarche, the association might attain statistical significance. If older age at menarche is inversely related to risk, however, such a finding would argue against lifetime estrogen exposure protecting against the development of HCC.

Age at natural menopause has been examined previously in the same five studies that examined age at menarche. Three of the studies found no association (La Vecchia *et al*, 1992; Tzonou *et al*, 1992; Kanazir *et al*, 2010), while one study found that later age at menopause increased risk (Mucci *et al*, 2001) and the other found that it decreased risk (Yu *et al*, 2003). The current study, in agreement with three of the prior five studies, found no association with HCC.

Prior findings suggested that use of MHT might reduce risk of HCC, as MHT has been inversely associated with fatty liver disease, liver enzyme levels and the development of diabetes in post-menopausal women (Clark *et al*, 2002; Kanaya *et al*, 2003; McKenzie *et al*, 2006). The current study, however, found a modest increased risk of HCC associated with MHT use (HR = 1.35), which was no longer statistically significant once adjustment was made for bilateral oophorectomy. In contrast, three prior studies of MHT reported inverse associations (Persson *et al*, 1996; Fernandez *et al*, 2003; Yu *et al*, 2003), while one small study from the US reported no association (Yu *et al*, 1991), though none of the studies reported adjustment for oophorectomy. It is conceivable that only certain MHT formulations reduce risk or that all formulations only reduce risk in a subset of women. For example, a study from Taiwan reported risk reductions only among women who were not virally infected (Yu *et al*, 2003). Unfortunately, information on specific MHT formulations was not available in the current study and the HBV/HCV status of most women could not be determined.

The other exogenous hormonal exposure of interest, OCs, has been more widely studied for an association with HCC than has MHT. Oral contraceptives were linked to the development of benign liver tumours in the 1970s, spurring studies of a relationship between OCs and HCC, which were begun in the early 1980s (Henderson *et al*, 1983). Although most studies of OCs and HCC have been limited by small sample sizes, IARC concluded in 1999 that there was sufficient evidence that OCs were hepatocarcinogenic, based on 11 studies. A formal meta-analysis of the same studies was conducted in 2007, however, and concluded that the evidence of a significant relationship was uncertain (Maheshwari *et al*, 2007). One additional study that was not included in the analysis (Yu *et al*, 2003), found no relationship between OC use and risk. The current study supports that conclusion as there was no evidence of association with either OC use or duration of use, even among women who had used OCs for five or more years. These findings may differ from those of prior studies in which a number of earlier studies limited their study populations to women

younger than age 50 years, whereas the majority of women in the current study were older than age 50 years. Thus, the current data argue that prior OC use is not linked to increased risk among women aged 50 years and older.

The current report is the largest study of reproductive factors and liver cancer or HCC conducted in the US. Other strengths include its wide geographic representation and its prospective design. In addition, sensitivity analyses that eliminated HCCs developing in the first years of follow-up, supported the results of the main analysis. Limitations, however, include that questions were asked in varying manners across studies and some data that would have been desirable to investigate, such as specific MHT formulations, were not able to be included. Other limitations included the inability to adjust the analysis for HBV and HCV infection status due to the limited availability of serum specimens, and the lack of information on pre-existing liver disease among the participants. In addition, only 20% of the women were younger than age 50 years at study enrolment and only 15% were non-white, so extrapolation of the findings to other groups of women should be done with caution.

The finding in rodent models that lower liver cancer risk among females may be due to oestrogenic inhibition of IL-6 production has stimulated interest in whether the same phenomenon might exist in humans (Naugler *et al*, 2007). Although the current study does not find a great deal of evidence to suggest that oestrogenic exposures throughout life reduce the risk of HCC in some women, the current study could not compare women known to have higher estrogen levels with women known to have lower levels.

In conclusion, the pooled analysis of data from 11 prospective US studies found the oophorectomy significantly increased the risk of HCC. Other reproductive variables, including OC use, were unrelated to risk. As the reproductive variables examined are only proxy measures of oestrogenic exposures, future studies that include serum measures of hormone levels may be able to provide further clarity on whether endogenous hormones increase risk of HCC.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

KAM was involved in study conception and design, analysis and interpretation of data, and study supervision. VVS contributed to analysis and interpretation of data. PTC was responsible for acquisition of data, analysis, and interpretation of data. BIG and JLP contributed to statistical analysis and interpretation of data. JC and LMS contributed to statistical analysis. MCA, GA, DAB, JEB, ATC, NDF, SMG, ARH, LH, LYK, JK, ML, JRP, JNP, MP, KR, CS, HDS, AS, JW-W, and AZ-J were involved in acquisition of data and critical reading of the manuscript.

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