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Colorectal Cancer in Northeast Italy: Reproductive, Menstrual and Female Hormone-related Factors

Silvia Franceschi, Ettore Bidoli, Renato Talamini, Salvatore Barra and Carlo La Vecchia

The role of reproductive and menstrual factors and a few medical conditions linked to female hormones in the aetiology of colorectal cancer was investigated in a case-control study conducted in Pordenone province in northeastern Italy, on 89 women with colorectal cancer and 148 controls admitted to hospital for a wide spectrum of acute, non-digestive nor neoplastic disorders. After adjustment for age and social class, parous women, as compared to nulliparous ones, were significantly protected against colorectal cancer (odds ratio, OR = 0.4, [95% confidence interval, CI:0.2–0.8]) and the risk appeared to decrease with successive pregnancies up to five or more (0.2, [0.04–0.6]). Compared to women who had their first birth at age 24 or less, the OR for those who had it at 30 or older was 2.0, but the inverse trend in risk was not significant. However, among parous women only, age at first birth, but not parity, seemed to retain a certain influence. Late age at menopause seemed to decrease colorectal cancer risk (OR for menopause at age ≥50 vs. <45 = 0.4, [0.2–1.0] χ^2_1 (trend) = 3.66). Conversely, age at last birth, number of abortions, years between marriage and first birth, age at menarche, pattern of menstrual cycle and occurrence of a few medical conditions potentially linked to female hormones were similarly reported by cases and controls. Due to the very limited number of oral contraceptive (OC) users (9 controls but only 1 case), and the lack of oestrogen replacement therapy users, the influence of exogenous female hormones on colorectal cancer could not be analysed meaningfully.
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INTRODUCTION

LONG-STANDING interest in the role of reproductive factors and female hormones in the aetiology of colorectal cancer stems from two major lines of evidence. Firstly, there are substantial similarities in the epidemiology of cancer of the colorectum and breast [1]. Incidence of and mortality from these neoplasms are positively correlated on both an international [2, 3] scale and within countries [4, 5]. Further, there are consistent and similar correlations between rates from these neoplasms and various indicators of modern affluence and/or intake of dietary fats and proteins [1, 6, 7]. Higher than expected incidence rates of colorectal cancer as well as breast, ovary and corpus uteri cancers have been noted in nuns [8], and in single women as compared to married women [9].

Secondly, a crossover of male and female incidence rates has been observed around menopausal age with male rates, formerly lower, exceeding female rates starting at age 55 [10]. Pregnancy has been suggested to account for such crossover phenomenon in colon cancer. In the incidence data of the Cancer Surveillance Program of Los Angeles county, for instance, the overall male-to-female ratios increased with age from roughly 0.8 to 1.6 whereas the male-to-never-pregnant women ratios remained virtually constant at approximately 0.8 [11].

Several investigations have dealt with the role of reproductive and menstrual factors and exogenous hormones [11–25] in the aetiology of colorectal cancer in women. A recent summary of most adequate studies [11] suggested an approximately 25% risk reduction in multiparous women (i.e. 3 or more live births).

To further elucidate this issue we analysed data from a multihypothesis case-control study on colorectal cancer conducted in Pordenone province, Friuli-Venezia Giulia Region, north-eastern Italy, a still partly rural area which went through a relatively late, but intensive process of industrialisation in the 1960s.

MATERIALS AND METHODS

Since June, 1987 we have been conducting a case-control study on colorectal cancer in the province of Pordenone. Trained interviewers identified and questioned patients who were (a) admitted for histologically confirmed colorectal cancer (and a wide variety of other conditions) to Aviano Cancer Center and all other local hospitals; (b) below age 75; and (c) permanent residents in Pordenone province. Approximately 2% of cases and 4% of controls refused to participate. The area under study is not covered by a cancer registry and it was not possible to estimate the proportion of colorectal cancer patients interviewed. The study hospitals, however, include all the diagnostic and therapeutical facilities available in the province, and therefore, the majority of cancer patients will have been referred there.

The present analysis is restricted to females, who represented 37% of interviewed individuals, and is based on 51 cases of colon cancer (median age = 58 years) and 38 of rectal cancer (median = 62), and 148 female hospital controls (median = 58), interviewed before June 1990. Of these controls, 14% were admitted for trauma, 30% for non-traumatic orthopaedic conditions, 20% for infections and acute surgical conditions, 20% for skin diseases and 16% for other illnesses such as ear, nose, throat or teeth disorders. Specifically excluded from the comparison group were patients whose diagnosis causing the current admission was for malignant, digestive or gynaecological disorders.

The questionnaire concerned sociodemographic indicators, related personal and medical history, reproductive and menstrual factors, personal habits such as smoking and frequency of consumption per week of alcohol and methylxanthine-containing beverages and 40 selected indicator foods.

For married women, social class is determined by their husband's occupation whereas single women (including divorcees and widows) are classified by their own occupation (Table 1).

Table 1. Distribution of 89 women with colorectal cancer and 148 controls according to age and various characteristics. Pordenone, Italy, 1986–1990

Variable*	Colorectal cancer	Controls
Age (yr)		
<50	17(19.1)	47(31.8)
50–59	24(27.0)	36(24.3)
60–69	28(31.5)	35(23.6)
≥70	20(22.5)	30(20.3)
Education (yr)		
≤4	29(32.6)	59(39.9)
5–7	45(50.6)	71(48.0)
≥8	15(16.9)	18(12.2)
Social class		
Professional and managerial occupations	7(7.9)	6(4.1)
Non-manual skilled occupations	25(28.1)	30(20.7)
Manual occupations	39(43.8)	85(58.6)
Farmers	18(20.2)	24(16.6)
Marital status		
Married	81(91.0)	137(92.6)
Never married	8(9.0)	11(7.4)
Body mass index		
≤23	30(33.7)	51(34.7)
24–26	26(29.2)	51(34.7)
≥27	33(37.1)	45(30.6)
Smoking status		
Never smoker	67(75.3)	115(77.7)
Ex-smoker	13(14.6)	15(10.1)
Current smoker	9(10.1)	18(12.2)
Family history of colorectal cancer		
No	85(95.5)	141(95.9)
Yes	4(4.4)	6(4.0)

No. (%)
*Some strata do not add up to the total because of missing values.

Various potential risk factors, plus age (in decades), and social class, were included in unconditional multiple logistic regression equations to obtain odds ratios (ORs), together with their 95% confidence interval (CI) [26]. The Generalised Linear Interactive Modelling (GLIM) [27] package was used to obtain maximum likelihood estimates and, for multiple levels of exposure, tests of significance for linear trend (assigned weights: 0, 1, 2, 3, 4). Since separate analysis of colon cancer and rectal cancer yielded very similar results, combined ORs are shown when not otherwise indicated. Likewise, the addition of various potentially confounding variables one at a time in the regression equations (i.e. education, marital status, smoking habit, body mass index and indicators of fat and vegetable consumption) left the ORs substantially unaltered. Only age and social class-adjusted ORs were, therefore, chosen for presentation.

RESULTS

Table 1 shows that colorectal cancer cases and controls were not significantly different as concerns age, education, social

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class, marital status and body mass index. Also the distributions of smoking habits and history of colorectal cancer in first-degree relatives were similar.

The distribution of colon and rectal cancer cases, separately, and control subjects according to reproductive and menstrual factors and corresponding ORs are given in Table 2. After allowance for age and social class, parous women, as compared to nulliparous ones, were significantly protected (OR = 0.4 [95% CI:0.2–0.8] $P<0.01$). The risk appears to decrease with successive pregnancies up to five or more (0.2 [0.04–0.6]), and the protective effect of pregnancy was present for both colon and rectal cancer (Table 2). Compared to women who had their first birth at age 24 or less, the point estimate for those who had it at 30 or older was 2.0, but the trend in risk was not statistically significant (Table 2).

Age at last birth, interval between marriage and first birth, as a possible indicator of subfertility, age at menarche, pattern of menstrual cycle and type of menopause did not seem to influence risk for either colon or rectal cancer in any noticeable way (Table 2). However, a late age at menopause seemed to decrease the risk of colorectal cancer: compared to women who had menopause at age 44 or less, those who reported it at age 50 or more showed an OR of 0.4 [0.2–1.0], and the inverse trend in risk was of borderline statistical significance ($\chi^2_1 = 3.66$).

The influence of a few medical conditions presumably related to female hormones (i.e. benign breast disease, benign ovarian tumours or cysts, uterine fibroids and thyroid disease) on colorectal cancer risk was also examined, but no significant associations emerged. Since only 9 control subjects and 1 case reported use of oral contraceptives, the apparent protective effect (OR = 0.2) had a very broad confidence interval [0.03–2.0]. No women had ever taken oestrogen replacement therapy.

Finally, in order to assess the joint effect of parity and age at first birth, the analysis was restricted to parous women (Table 3). The effect of age at first birth seemed to be small but consistent in the two strata of parity examined. Conversely, after allowing for the effect of age at first birth, women with three or more children were not apparently at increased risk as compared to those with one or two only.

DISCUSSION

The present study lends further support to the possibility that having children and, perhaps, being young when the first child is born are protective against the development of colorectal cancer. At least 13 case-control studies [11–14, 16–20, 22–25] and one prospective study [21] have provided data on the role of reproductive and menstrual factors in colorectal cancer aetiology: with three exceptions [17,19,23], all showed a protective effect, although limited, for parity, while in three of them [18, 20, 22] a favourable effect of early age at first pregnancy or birth was also noted. The protection from multiparity shown in the present study is among the strongest ever reported and the direct association of colorectal cancer risk with age at first birth, although not significant, is also compatible with the findings of those [18, 20, 22] who suggested that the timing of full-term pregnancies may have a role. In fact, a look at the joint influence of number of births and age at first birth, although based on small numbers, suggests that, after allowance for mutual confounding effect, age at first birth but not parity remained associated with an elevated risk of colorectal cancer (Table 3).

Several mechanisms have been hypothesised to explain the effect of full-term pregnancies and, perhaps, age at first birth on the development of colorectal cancer [10, 28], often taking

Table 2. Relation between reproductive and menstrual factors and colorectal cancer

Variable*	Cases: Colon	Cases: Rectal	Controls	OR† (95% CI)
No. of births				
0	12	9	15	1‡
1	4	5	11	0.6(0.2–1.9)
2	20	8	59	0.4(0.2–1.0)
3–4	12	11	39	0.4(0.2–0.9)
≥5	1	3	14	0.2(0.04–0.6)
χ^2_1(trend)				8.56, $P=0.003$
Age at first birth (yr)				
<25	14	14	73	1‡
25–29	18	9	41	1.7(0.8–3.3)
≥30	5	4	9	2.0(0.7–5.9)
χ^2_1(trend)				2.79, $P=0.09$
Age at last birth (yr)				
<30	12	9	50	1‡
30–34	17	9	40	1.3(0.6–2.8)
≥35	8	9	33	0.8(0.3–1.8)
χ^2_1(trend)				0.30, $P=0.58$
Number of abortions				
0	33	27	106	1‡
≥1	16	9	32	1.4(0.7–2.6)
Years between marriage and first birth				
<1	6	12	41	1‡
1–2	22	13	64	1.4(0.7–2.8)
≥3	9	1	15	1.6(0.6–4.4)
χ^2_1(trend)				1.00, $P=0.32$
Age at menarche (yr)				
≤12	14	9	51	1‡
13–14	25	19	61	1.7(0.9–3.3)
≥15	11	10	35	1.2(0.6–2.6)
χ^2_1(trend)				0.38, $P=0.54$
Menstrual cycles				
Regular	41	32	125	1‡
Irregular	10	5	23	1.3(0.6–2.8)
Age at menopause (yr)				
<45	8	7	12	1‡
45–49	14	12	31	0.7(0.3–1.8)
≥50	14	16	57	0.4(0.2–1.0)
Premenopausal				
χ^2_1(trend)	15	3	47	0.6(0.2–2.0)
				3.66§, $P=0.06$
Type of menopause§				
Natural	30	29	88	1‡
Artificial	5	6	14	1.4(0.6–3.3)

*Some strata do not add up to the total because of missing values.
†Estimates from multiple logistic equations adjusting for age and social class.
‡Reference category.
§Postmenopausal women only.

Table 3. Odds ratios of colorectal cancer for parity in various strata of age at first birth*

	Age at first birth (yr)		
	<25	≥25	All
Parity			
≥3	1†	1.8(0.7–4.7)	1†
1–2	1.1(0.4–2.7)	1.9(0.8–4.5)	1.1(0.6–2.1)
All	1†	1.8(0.9–3.4)	

*Adjusted for age, social class, parity or age at first birth by means of the Mantel–Haenszel procedure.
†Reference category.

breast cancer as a model. Modifications of oestrogen profile caused by pregnancies and, possibly, their effects on bile metabolisms [10] have been considered, but immunological effects of pregnancies [12], increased physical activity associated with large families [21], and as yet unidentified lifestyle factors associated with having children [22] have also been proposed, especially since a "parity" effect has also been observed in male patients with colorectal cancer in some [21, 22, 29], but not all [18], previous studies.

Most previous investigators did not report their data in sufficient detail to study the effect of each successive pregnancy, but Peters *et al.* [11] suggested an U-shaped relationship between pregnancies and colon cancer risk, with risks decreasing down to four full-term pregnancies and then increasing again. In this partly rural Italian population, a relatively high proportion of multiparous women was found and a beneficial effect seemed to derive also from full-term pregnancies following the first three. Absolute numbers of cases and controls were, however, limited and it is possible that the apparently greater strength of the association in the present study is due to the play of chance.

Among other reproductive and menstrual factors the only one which seems, from the present study, to deserve further attention is age at menopause. A weak protective effect for a late age at menopause emerges from this as well as previous investigations [11, 19, 21]. This finding, if not due to chance, is in conflict with the breast cancer model and is difficult to interpret, in the light of the crossover in male and female age-specific rates of colon cancer around age 55.

The lack of association with age at menarche is compatible with most previous work [11, 21], as well as the apparent (although non-significant) protection given by oral contraceptives, inversely associated with colorectal cancer risk in some [25], but not all [16], previous investigations. Similarly none of the medical conditions potentially linked with the female hormones herein considered seems to affect colorectal cancer risk.

In respect to potential distortions of present results, recall or information biases probably do not affect greatly the reproductive and menstrual variables herein considered. In order to avoid selection bias, a great effort was made to contact all cases of colorectal cancer and eligible controls admitted to the collaborating hospitals and to interview them as soon as possible after diagnosis. As a results of such procedure, very few subjects had to be excluded because of lack of histopathological material, early discharge, death or severe illness or refusal to collaborate. A good comparison of cases and controls should also have been achieved by interviewing controls with a broad spectrum of conditions and coming from the same catchment area of cases. Indeed, control subjects would have been referred, if afflicted by colorectal cancer, to the hospitals where cases were contacted. In relation to confounding the role of parity, age at first birth and age at menopause persisted after allowance for major potential confounders, including social class, education and consumption of a few food indicators significantly associated with risk of colorectal cancer (i.e. eggs and various types of vegetables, data not shown). Unfortunately no information was available on physical activity.

In conclusion, of some concern is, as usual, the hospital-based design of the present investigation, whose strengths are, however, the almost complete response rate, the similar catchment area of cases and controls, the comparability of medical histories and the agreement with studies conducted in very different contexts, chiefly North American areas.

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Paired Tumour Infiltrating Lymphocyte (TIL) and Tumour Cell Line from Bladder Cancer: a New Approach to Study Tumour Immunology *in vitro*

A.M.E. Nouri, A. Bergbaum, E. Lederer, D. Crosby, A. Shamsa and R.T.D. Oliver

This paper reports the first example of tumour infiltrating lymphocytes (TILs) and a tumour cell line from the same individual and analyses their characteristics. The tumour cell line (CAT), derived from a patient with well-differentiated (G3pTa) TCC, has been in culture for 24 months and subcultured more than 100 times. Epithelial origin was established by electronmicroscopy and use of a range of monoclonal antibodies (Mabs) against cytokeratins. The TILs isolated from the same tumour expressed all the phenotypic characteristics of normal activated T cells and demonstrated low levels of cytotoxicity against the autologous tumour line (CAT). Comparison of cell surface molecules of these cells revealed the loss of HLA-B7, B44 and Bw6 from the CAT cells whilst maintaining HLA-A2, A3 and Bw4. Karyotypic analysis demonstrated three rearranged chromosomes (between chromosomes 4 and 11, 10 and 13, 11 and 17) on CAT cells. The potential that study of paired autologous tumour cells and TILs in culture offers for studying the role of MHC antigens in tumour rejection and the impact of different approaches to correcting the defect are reviewed.
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INTRODUCTION

IT IS now well established from several animal models [1–4] that transfection of a major histocompatibility complex (MHC) class I gene into a tumour cell line that had lost these antigens during malignant transformation results in loss of tumorigenicity. In addition, in some cases, immunisation of animals with the class I transfected tumour cells has produced complete resistance to the original class I negative tumour cells [1]. This, taken with increasing evidence for loss of class I antigen expression on human tumours, has led to increased interest in the role of MHC antigens in tumour rejection [2–4].

For several human tumour types there has been a correlation between the degree of tumour infiltration by lymphocytes and prognosis [5, 6]. Prompted by the observation from our own studies demonstrating a correlation between the degree of class I loss and difficulty of expanding tumour infiltrating lymphocytes using interleukin-2 (IL-2) (A.M.E.N. *et al*) and the report of Aebersold *et al.* [7] who demonstrated that the TILs marked with the gene for neomycin phosphotransferase homed to tumour sites of melanoma patients that were undergoing rejection, an attempt was made to culture tumour cell and TILs from the same patient with the long-term aim to investigate the influence of MHC antigen expression on TIL function *in vitro*.

This paper reports the first example of successful establishment of a bladder tumour cell line and TILs from the same patient and compares their oncogene activation, karyotype and cell surface markers including MHC class I and II antigens.

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