The genesis and progression of colon cancer is complex. The role of estrogen, progesterone and androgens on the development of colonic cancer has received much attention and they may play a role in colonic carcinogenesis. The mechanisms by which hormones regulate colon cancer growth are extremely complex. They may act directly on target cells or indirectly through the release of other autocrine, paracrine or endocrine agents (Goustin et al. 1986, Milsom 1993). These hormones may exert their tumor-modulating effects indirectly through interaction with a nuclear DNA sequence termed 'hormone responsive element' which regulates transcription of an RNA sequence controlling growth factor binding at the cell surface. In addition, the presence of sex steroid hormone receptors in patients with colonic cancer have been demonstrated by several investigators (McClendon et al. 1977, Alford et al. 1979, Sica et al. 1984, Francavilla et al. 1987) and in the previous chapter, supports the hypothesis that the growth of colon/rectal tumors may be influenced
by steroid hormones.

In view of the above findings, the aim of the present study was to investigate the role of circulating levels of estradiol, progesterone, testosterone and its precursor dehydroepiandrosterone sulfate in patients with colon/rectal cancer at diagnosis. Additionally, the levels of steroid hormones were correlated with clinicopathologic parameters and steroid receptors.

STUDY DESIGN

Patients

In this study, a total of 60 male patients with Dukes B or C colon/rectal carcinoma, registered at The Gujarat Cancer and Research Institute, Ahmedabad, between January 1989 and December 1990 were enrolled.

The details of clinical history were noted from the case files maintained at the Medical Record Department of the Institute and recorded at the Division of Molecular Endocrinology. The clinical history included age, anatomic site of the lesion (colon/rectum), operative findings, Dukes stage, histologic type and grade of the tumor.
The reference group consisted of 30 healthy (nonsmokers and smokers) age-matched controls.

Disease was staged according to modified Dukes classification (Dukes and Bussey 1958). The histologic characteristics were assessed independently by two histopathologists.

Blood collection
Venous blood samples were collected pretherapeutically, between 9:00 and 11:00 AM to avoid diurnal variation. Blood was separated within 2 hours, and serum was aliquoted and preserved at -70°C till analysis.

Steroid hormone assay
Serum estradiol (E), progesterone (Pg) and testosterone (T) were assayed in 60 patients using radioimmunoassay (RIA) kits procured from Binax, USA. Dehydroepiandrosterone sulfate (DHEA-S) was determined in 55 patients using RIA kits procured from Diagnostic Systems Laboratories Inc., USA. The assays were performed in duplicate with an intra- and inter-assay coefficient of variation (CV) of 3% to 8%, along with internal quality controls. The sensitivity of the assay kit was 8.0 pg/ml for E, 0.05 ng/ml for Pg, 0.11
ng/ml for T and 10.0 ng/ml for DHEA-S. The normal range of steroid hormones is listed below:

<table>
<thead>
<tr>
<th>Hormone/precursor</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) E</td>
<td>6.00 to 36.00 pg/ml serum.</td>
</tr>
<tr>
<td>(2) Pg</td>
<td>Undetectable to 0.85 ng/ml serum.</td>
</tr>
<tr>
<td>(3) T</td>
<td>3.00 to 9.00 ng/ml serum.</td>
</tr>
<tr>
<td>(4) DHEA-S</td>
<td>840.0 to 4000.00 ng/ml serum.</td>
</tr>
</tbody>
</table>

The levels above or below the normal range were considered elevated or subnormal, respectively.

Statistical analysis
Significance was calculated using an exact contingency table test for order data and Fisher's two sided exact test (Mehta and Patel 1983). P values less than 0.05 were considered significant. Correlation between two parameters was calculated using Pearson's correlation coefficient (r) method.

RESULTS

Incidence of steroid hormones
In patients with colon/rectal cancer the preoperative levels of E (P<0.02) and Pg (P<0.0001) were significantly higher while the levels of T (P<0.0001)
and DHEA-S (P<0.0001) were significantly lower as compared to their respective controls (Table 1). The levels of E2 and Pg were elevated in 38% (23/60) and 18% (11/60) patients, respectively. Thirty-five percent (21/60) and 49% (27/55) patients had low levels of T and DHEA-S, respectively. Elevated levels of DHEA-S were observed in 2% (1/55) patients. The ratio of T:E2 was significantly lower (P<0.0001) in patients with colon/rectal cancer than that of controls (Table 1).

Table 1: Distribution of steroid hormones in controls and in patients with colon/rectal cancer (M ± SE).

<table>
<thead>
<tr>
<th>Hormones/ precursor</th>
<th>N</th>
<th>Controls</th>
<th>N</th>
<th>Colon/rectal cancer patients</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2 (Pg/ml)</td>
<td>30</td>
<td>23.35± 1.65♀</td>
<td>60</td>
<td>41.23± 6.97♀</td>
<td>0.00-260.00</td>
</tr>
<tr>
<td>Pg (ng/ml)</td>
<td>30</td>
<td>0.07± 0.02#</td>
<td>60</td>
<td>0.62± 0.13#</td>
<td>0.00-4.40</td>
</tr>
<tr>
<td>T (ng/ml)</td>
<td>30</td>
<td>6.47± 0.23$</td>
<td>60</td>
<td>3.73± 0.23$</td>
<td>0.48-8.20</td>
</tr>
<tr>
<td>DHEA-S (ng/ml)</td>
<td>20</td>
<td>2321.66±192.44♂</td>
<td>55</td>
<td>1009.18±110.39♂</td>
<td>55.70-4351.80</td>
</tr>
<tr>
<td>T:E2 ratio</td>
<td>30</td>
<td>314.44± 27.81♀</td>
<td>60</td>
<td>133.72± 19.44♀</td>
<td></td>
</tr>
</tbody>
</table>

P values: #,$,®,,+ P<0.0001
* P<0.02
Relation of steroid hormones to age

In younger patients (age <40 years), the levels of Pg and DHEA-S were higher than that observed in older (age >40 years) patients. However, the difference was statistically non-significant. The distribution of E\textsuperscript{2} and T was similar in younger and older patients (Table 2).

Table 2: Relation of steroid hormones to age of the patients (N ± SE).

<table>
<thead>
<tr>
<th>Hormones/precursor</th>
<th>N</th>
<th>Age &lt;40 Years</th>
<th>N</th>
<th>Age &gt;40 Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>E\textsuperscript{2} (pg/ml)</td>
<td>13</td>
<td>46.33 ± 18.42</td>
<td>47</td>
<td>39.82 ± 7.92</td>
</tr>
<tr>
<td>Pg (ng/ml)</td>
<td>13</td>
<td>1.05 ± 0.37</td>
<td>47</td>
<td>0.50 ± 0.13</td>
</tr>
<tr>
<td>T (ng/ml)</td>
<td>13</td>
<td>3.59 ± 0.57</td>
<td>47</td>
<td>3.77 ± 0.26</td>
</tr>
<tr>
<td>DHEA-S (ng/ml)</td>
<td>12</td>
<td>1456.04 ± 291.48</td>
<td>43</td>
<td>884.47 ± 107.99</td>
</tr>
</tbody>
</table>

Relation of steroid hormones to anatomic site of the lesion

A trend of higher level of E\textsuperscript{2}, Pg and T was observed in patients with cancer of the rectum than those with colon.
cancer. The levels of DHEA-S were found to be similar between the two subgroups (Figures 1-4).

Relation of steroid hormones to Dukes stage
The levels of E and Pg were elevated in 44% (14/32) and 16% (5/32) patients with Dukes B disease, and 32% (9/28) and 21% (6/28) patients with Dukes C disease, respectively. Subnormal levels of T and DHEA-S were noted in 38% (12/32) and 52% (16/31) patients with Dukes B disease, and 32% (9/28) and 46% (11/24) patients with Dukes C disease, respectively. Moreover, significant intergroup variation in the mean level of each hormone was not observed (Figures 1-4).

Relation of steroid hormones to histologic type of the tumor
As the number of patients with squamous cell carcinoma and transitional cell carcinoma were few (N=4), the data was not analysed statistically. However, it was noted that the mean level of E was higher while the levels of T and DHEA-S were lower in patients with adenocarcinoma than in patients with squamous cell carcinoma and transitional cell carcinoma. The distribution of Pg was similar in the histologic types (Figures 1-4).
Relation of steroid hormones to histologic grade of the tumor

The mean DHEA-S level was lower in patients with grade I tumor than those with grade II or III tumors but the difference was statistically significant only between grade I and II tumors (P<0.05). Further, the levels of E and Pg showed a decreasing trend from grade I to grade III whereas the level of T was similar in patients with grade I, II and III tumors (Figures 1-4).

Figure 1: Histogram showing distribution of E₂ in patients with colon/rectal cancer according to clinicopathologic parameters. C-colon; R-rectum; A-adenocarcinoma; O-others; W-well differentiated; M-moderately differentiated; P-poorly differentiated.

:200:
Figure 2: Histogram showing distribution of Pg in patients with colon/rectal cancer according to clinicopathologic parameters. C-colon; R-rectum; A-adenocarcinoma; O-others; W-well differentiated; M-moderately differentiated; P-poorly differentiated.
Figures 3 and 4: Histogram showing distribution of T and DHEA-S in patients with colon/rectal cancer according to clinicopathologic parameters. C-colon; R-rectum; A-adenocarcinoma; O-others; W-well differentiated; M-moderately differentiated; P-poorly differentiated.
Correlation of circulating estradiol (E\textsubscript{2}) and progesterone (Pg) to steroid receptors

Circulating levels of E\textsubscript{2} and Pg when correlated with steroid receptors, a weak inverse correlation was observed between E\textsubscript{2} and ER (r=-0.14; Figure 5), E\textsubscript{2} and PR (r=-0.16; Figure 6), Pg and ER (r=-0.113; Figure 7), and Pg and PR (r=-0.097; Figure 8).

Figure 5: Scatterogram showing the distribution of E\textsubscript{2} and ER in patients with colon/rectal cancer. A weak inverse correlation was observed between them.
Figure 6: Scatterogram showing the distribution of $E_2$ and PR in patients with colon/rectal cancer. A weak inverse correlation was observed between them.
Figure 7: Scatterogram showing the distribution of Pg and ER in patients with colon/rectal cancer. A weak inverse correlation was observed between them.
Figure 8: Scatterogram showing the distribution of Pg and PR in patients with colon/rectal cancer. A weak inverse correlation was observed between them.
In addition, a trend of higher circulating level of $E_2$ was noted in patients with ER- or PR- tumors than their respective counterparts. Moreover, the distribution of $Pg$ was similar in ER+, ER-, PR+ and PR- subgroups (Table 5).

Table 5: Distribution of $E_2$ and $Pg$ according to steroid receptors ($M \pm SE$).

<table>
<thead>
<tr>
<th>Receptors</th>
<th>N</th>
<th>$E_2$ (pg/ml serum)</th>
<th>$Pg$ (ng/ml serum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER+</td>
<td>27</td>
<td>36.42 ± 8.51</td>
<td>0.68 ± 0.21</td>
</tr>
<tr>
<td>ER-</td>
<td>29</td>
<td>49.00 ± 12.10</td>
<td>0.64 ± 0.18</td>
</tr>
<tr>
<td>PR+</td>
<td>31</td>
<td>33.56 ± 7.53</td>
<td>0.74 ± 0.20</td>
</tr>
<tr>
<td>PR-</td>
<td>25</td>
<td>54.56 ± 14.07</td>
<td>0.56 ± 0.18</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Epidemiologic and metabolic studies have suggested a relation of reproductive events and exogenous and endogenous sex hormones to female colon/rectal cancer (McMichael and Potter 1980). It has also been suggested that the association of breast and colorectal cancer in women is a result of shared reproductive hormonal
factors. The role of steroid hormones in men with colon/rectal cancer has been largely unexplored. The present study indicated that patients with colon/rectal cancer had dual steroidal abnormalities as compared to controls i.e. higher levels of circulating E and Pg with concomitant low levels of T and DHEA-S. One of the possible explanation for elevated E levels might be due to the activity of sex-steroid conversion enzyme aromatase, which converts C to C steroids such as E and estrone. Similar observation has been noted in patients with pancreatic cancer (Corbishley et al. 1986). The reason for higher serum Pg in these patients is not known. The observed low circulating levels of T in patients as compared to controls were comparable to the reports of several investigators in different malignancies (Labhart 1974, Greenway et al. 1983, Recchione et al. 1983, Corbishley et al. 1986, Inutsuka et al. 1986, Andersson et al. 1993, Bhatavdekar et al. 1994 a,b,c). The exact cause of low circulating levels of T at the time of diagnosis in cancer patients remains obscure. However, it has been suggested that in patients with gastric cancer, testosterone was absorbed or destroyed in cancer tissues or cancer cells by a certain mechanism or that testosterone was excreted into
the lumen of digestive tract (Inutsuka et al. 1986). A similar mechanism might be operating in patients with colon/rectal cancer. In addition, the study demonstrated low circulating levels of DHEA-S—a main adrenal androgen precursor—that is converted to testosterone both in testis and extraglandular tissues (Labhart 1974, Recchione et al. 1983, Wilkinson 1987, Milsom 1993). The low levels of DHEA-S might be due to adrenocortical insufficiency, which subsequently might have resulted in low concentration of T. Moreover, the ratio of T:E was significantly low in patients with colon/rectal cancer when compared to controls, probably suggestive of an imbalance between androgens and estrogens.

Steroid hormones when correlated with age and clinicopathologic parameters, significant intergroup variation was not obtained except for grade of the tumor. Patients with grade I tumors exhibited significantly low concentration of DHEA-S than grade II tumors.

It is well known that circulating E and Pg may, by way of several mechanisms, affect the apparent concentrations of ER and PR (Vihko and Isotalo 1981).
Vihko et al. (1980) found a positive correlation of circulating E with ER and PR in patients with breast cancer. The present study reported a weak inverse correlation between circulating E and Pg, and steroid receptors. The levels of E were found to be lower in patients with ER+/PR+ tumors.

From these results it may be concluded that steroid hormones may play an important role in the etiology of colon/rectal cancers in terms of individual susceptibility at the cellular level by altering receptor function or metabolism. Thus, steroid hormonal abnormalities may precede and presumably favour the onset of colon/rectal cancer and the altered hormone levels might be responsible for the pathogenesis of colon/rectal cancer.

A B S T R A C T

This chapter describes the significance of circulating steroid hormones (N=60) such as estradiol (E), progesterone (Pg) and testosterone (T) and androgen precursor dehydroepiandrosterone sulfate (DHEA-S; N=55) in patients with colon/rectal cancer. In these patients, levels of E (P<0.02) and Pg (P<0.0001) were
significantly higher whereas the levels of $T$ ($P<0.0001$) and $DHEA-S$ ($P<0.0001$) were significantly lower in colon/rectal cancer patients than their respective controls. A trend towards higher levels of $Pg$ and $DHEA-S$ was noted in younger patients (age <40 years) than in older patients (age >40 years) while the distribution of $E$ and $T$ was similar in younger and older patients. The mean level of $DHEA-S$ was significantly lower in grade I tumors than in grade II tumors ($P<0.05$) whereas such a difference was not observed for $E$, $Pg$ and $T$. Significant differences were not observed when steroid hormone levels were correlated with anatomic site of tumor, Dukes stage and histologic type of tumor.

A weak inverse correlation was observed between ER and $E$ ($r=-0.14$) and between PR and $Pg$ ($r=-0.097$).
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