# Predominance of Younger Age, Advanced Stage, Poorly-differentiated and Mucinous Histology in Israeli Arab Patients with Colorectal Cancer 

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#### Abstract

Background: Colorectal cancer (CRC) incidence in the Israeli population is higher in the Jewish population than among Arabs. Materials and Methods: To determine the differences in demographic, clinical, histopathological and molecular characteristics of CRC between these two ethnic groups, 125 Arab patients treated at 3 community hospitals over a 20-year period were compared to a group of 208 consecutive Jewish patients. The mutator (replication error-positive [RER]) phenotype was detected by immunohistochemical evaluation of hMLH1 and hMSH2 protein expression in tumor tissue. Results: The Arab patients were younger than the Jewish patients with a higher percentage of poorly-differentiated and mucinous cancers and a higher percentage of advanced stage cancers (Dukes' $C+D$ ) at presentation. The mutator phenotype was detected at similar rates in both ethnic groups. Conclusion: Our study demonstrated that CRC patients from two major ethnic populations in Israel, Arabs and Jews, differed in terms of the prevalence of the disease, pathological features and age at presentation, but not in frequency of mismatch-repair-positive cancers.


Colorectal cancer (CRC) is the third leading cancerrelated cause of mortality in the adult population of Israel. The prevalence of CRC in the Israeli population varies widely. One of the most important factors that

[^0]influence this variability is the ethnic origin of patients. Data from the Israel Cancer Registry (ICR) show that the Israeli Arab population has a substantially lower incidence of CRC in all age groups compared to the Jewish population: according to these data, less than $5 \%$ of all CRC cases in 2002 were patients of Arab origin, although the Arab sector comprises $20 \%$ of the entire population of Israel (1-3).

The evolution of CRC is linked to a close interrelation of genetic and environmental factors. The Jewish and Arab communities reside within a relatively small geographical area, thus being exposed to common environmental and ecological factors. However, the two ethnic communities differ in socioeconomic status as well as in many cultural and nutritional characteristics and genetic background. Most Israeli Arabs reside in rural areas and are occupied in physical and agriculture work. The Arab population mainly consists of large families who have been living a non-migrant lifestyle for many years. In contrast, the Jewish population is much more heterogeneous with a mixture of native Israelis and Jews from diverse diasporas, designated as Sephardi and Ashkenazi, with intra-ethnic variability in CRC prevalence and CRC-related mortality. In spite of this intra-ethnic variability between Ashkenazi and Sephardic Jews, there is a substantial difference in the prevalence of CRC between Jews and Arabs in Israel.

Two major genetic pathways of evolution of CRC are the microsatellite instability (MSI) pathway, also called replication error-positive, and the chromosomal instability pathway (CIN) (4). MSI differs from CIN by right side predominance, mucinous or poorly-differentiated histology and younger age of onset $(5,6)$. Most individuals with MSIpositive sporadic and familial CRC have defective function
in two mismatch repair (MMR) genes: hMLH1 and hMSH2 (7, 8). Approximately $10-20 \%$ of non-familial CRC are characterized by MSI (7, 9). The MSI phenotype is usually detected by evaluation of microsatellite markers in tumor tissue, although several studies have demonstrated that immunohistochemistry correlates highly with defective MMR function and is highly predictable for the evaluation of MMR status in these tumors $(10,11)$.

There are limited data on the clinicopathological and molecular characteristics of CRC in different ethnic populations in Israel. The reported variability in the prevalence of CRC between Arab and Jewish populations in Israel prompted us to assess the demographic and clinicopathological differences and patterns of replication error phenotype in tumors in a cohort of Israeli Arab patients with CRC and to compare these data to Jewish CRC patients.

## Materials and Methods

One hundred and twenty-five Israeli Arab patients with CRC, treated over a 20-year period (1985-2004) at the surgical departments of 3 community-based academic hospitals, were enrolled in the study. The cases were obtained by review of pathological and clinical records and the controls were obtained from a computerized list of Israeli Jewish patients treated at one of the hospitals over a 5 -year period (2000-2004). The AstlerColler modified Dukes' classification was used for staging of the tumors.

Data were collected on demographic characteristics, including age, sex, area of residence (town or rural), histomorphological characteristics of CRC, staging, tumor site and the expression of MMR proteins. The border between proximal and distal tumor location was the splenic flexure. Staging for synchronous tumors was shown for the higher stage tumor only.

Immunohistochemistry. Formalin-fixed, paraffin-embedded tumor samples were used for immunohistochemical staining. For the evaluation of MMR protein expression status, two mouse monoclonal antibodies against MLH1 or MSH2 protein (clone G168-15 [B\&D, PharMingen, San Diego, CA, USA] at dilution 1:70 and clone FE-11 [Zymed Lab, Inc., San Francisco, CA, USA] at dilution 1:150, respectively) were used.

First, $5-\mu \mathrm{m}$ thick sections of each paraffin block were stained with hematoxylin and eosin to choose representative tumor samples. The avidin-biotin-peroxidase method was used for immunohistochemical staining. Three to four $5-\mu \mathrm{m}$ thick sections were used for each antibody. For staining, the sections were applied on sialine-coated slides, deparaffinized in xylene and rehydrated by using graded alcohol solutions. Endogenous peroxidase activity was blocked by incubation in hydrogen peroxide. A microwave oven was used for antigen retrieval. Nonspecific binding sites were blocked with $10 \%$ non-immune serum, followed by incubation of the slides with the appropriate primary antibodies as follows: one hour at room temperature for hMLH1 monoclonal antibody (clone G168-15, at dilution 1:70) and overnight at $4^{\circ} \mathrm{C}$ for hMSH2 (clone FE-11, at dilution 1:150). After
several washes, sections were incubated with biotinated Ig against mouse or rabbit IgG for 10 min at room temperature using a Histatstin-Plus kit (Zymed Laboratories, Inc.). After repeated washing in phosphate buffer solution, the sections were incubated for 10 min with streptavidin-peroxidase complex. Staining was developed with AEC (3-amino-9- ethylcarbasole) for 5-15 min and counterstained with Meyer's hematoxylin.

Tumors were recorded as positive for either MLH1 or MSH2 if tumor cells showed distinct nuclear staining in the presence of an appropriate positive control. Normal adjacent colonic mucosa and lymphoid follicles served as an internal control for positive immunostaining. Staining by omitting the primary antibody served as a negative control. Only those tumors with technically appropriate staining for both MLH1 and MSH2 antibodies were included in the study analysis.

Statistical analysis. The Student $t$-test and the $\chi^{2}$ test were used as appropriate for the evaluation of statistical significance in both population groups. Statistical analysis was performed by using the statistical software package SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

## Results

Demographics. Clinical and pathological data from 125 Israeli Arab patients and 209 Israeli Jewish patients were analyzed in our study. The main demographic, clinical and pathological data of the patients are provided in Table I. Eighty-five percent of the Arab patients resided in rural areas as compared to only $15 \%$ of the Jewish patients. The Arab patients were younger than the Jewish patients at mean ages of 62.9 and 74 years, respectively ( $p<0.001$ ). Fifteen percent of the Arab and $1.4 \%$ of the Jewish patients were under age 50 .

Location and pathology of CRC. Proximal and distal colon cancer were found with the same frequency in both patient groups. A lower percentage of tumors in the descending colon and a slightly higher percentage of rectal cancers were detected among the Arab patients, although this difference was not statistically significant (Table I).

The majority of tumors in both ethnic groups were wellto moderately-differentiated, while the Arab patients had a significantly higher percentage of poorly-differentiated and mucinous tumors $(p<0.01)$. Fifty percent of the Arab patients presented with locally advanced or metastatic (Dukes' $\mathrm{C}+\mathrm{D}$ ) stage as compared to $32 \%$ of Jewish patients $(p=0.02)$.

Expression of MMR proteins. Sixty-five tumors from Arab and 110 tumors from Jewish patients were stained for MMR proteins (Table I). Defective protein expression patterns with loss of nuclear staining in the tumor tissue for either hMLH1 or hMLH2 were detected in 13/65 (20\%) tumor samples from the former patients and in 12/110 (11\%) tumor samples from the latter patients $(p=0.1)$.

Table I. Comparison of clinical and pathological features between youngonset and late-onset patients with colorectal cancer.

| Parameter | Jews No. (\%) | Arabs No. (\%) | $P$ |
| :---: | :---: | :---: | :---: |
| No. of patients | 208 | 125 |  |
| Mean age (range) | 74 | 62.9 | <0.001 |
| Young (age <50) | 3 (1.4\%) | 16 (15\%) | <0.001 |
| Gender |  |  |  |
| Male | 117 | 62 | NS |
| Female | 91 | 61 |  |
| M:F | 1:0.78 | 1:1.1 |  |
| Dukes' stage\# |  |  | $0.002^{\text {\#\# }}$ |
| A | 5 (2.4) | 0 |  |
| B | $36+95$ (63) | $8+49$ (45.2) |  |
| C | $8+50$ (27.9) | $8+46$ (43.7) |  |
| D | 14 (6.7) | 14 (11.1) |  |
| Tumor location ${ }^{\dagger}$ |  |  |  |
| Right (cecum + ascend) | 53 (24.9) | 33 (25.9) | N.S. |
| Transverse | 15 (7) | 9 (7.8) |  |
| Descending | 50 (23.5) | 16 (12.5) |  |
| Sigmoid | 62 (29.1) | 43 (33.8) |  |
| Rectum | 33 (15.5) | 26 (20) |  |
| Synchronous tumors | 5 | 2 | N.S |
| Differentiation ${ }^{\dagger}$ |  |  | $<0.01$ |
| Good | 40 (18.8) | 36 (28.3) |  |
| Moderate | 154 (72.4) | 67 (52.7) |  |
| Poor | 13 (5.8) | 12 (9.5) |  |
| Mucinous | 6 (3) | 12 (9.5) |  |
| MMR status |  |  |  |
| No tumors evaluated | 110 | 65 | N.S. |
| Positive | 12 (11) | 13 (20) |  |
| Negative | 98 (89) | 51 (80) |  |

\#Astler-Coller's modified Dukes' staging: Dukes' Stage $B=(B 1+B 2)$ Dukes' Stage C $=(\mathrm{C} 1+\mathrm{C} 2)$
\#\# $p$ value stands for early (Dukes' $\mathrm{A}+\mathrm{B}$ ) vs. advanced (Dukes' $\mathrm{C}+\mathrm{D}$ ) stages
$\dagger$ shown as number of tumors

## Discussion

The demographic and clinicopathological characteristics and expression of MMR proteins were compared in a cohort of Arab and Jewish patients with CRC, being representative of two major ethnic groups of the Israeli population. Arab patients showed a different demographic and clinicopatholgical profile of cancer, with lower prevalence of the disease, and a higher percentage of younger patients, "unfavorable" histological types and patients in advanced stages of the disease. No difference in the rate of MMR-positive tumors was detected among the Arab patients. To the best of our knowledge, this is the largest series published to date on this topic.

The Israel Cancer Registry (ICR) provides a single nationwide resource of data on the prevalence and demographic characteristics of CRC in different ethnic subpopulations in Israel. These data show that Arab patients display a significantly lower prevalence of CRC. In fact, the age-standardized ratio of colon cancer per 100,000 in year 2002 was 29 for males and 24 for females among Jewish $v s$. 12.5 for males and 15 for females in non-Jewish (mostly Arab) populations (1). Furthermore, data on age-related differences of colon cancer show a higher percentage of young Arab patients as compared to Jewish patients $(1,12)$. Comparable data on the incidence and age distribution of CRC in Arab populations of neighboring countries have been published by the Middle East Cancer Consortium (1315). Similar to the ICR, our results showed a significantly younger age at presentation among Arab patients compared to Jewish patients: the average age of Arab patients, being on average 10 years less than the Jewish patients, with $15 \%$ of Arab patients being younger than age 50 as compared to only $1.5 \%$ among Jewish patients.

The Jewish and Arab communities reside in relatively close proximity and have a common geographic and ecological milieu, yet differ substantially in many social, cultural and nutritional characteristics. A higher percent of Arabs reside in rural areas and are involved in physical work (12). Substantial differences have been reported in dietary habits between the two ethnic populations, with higher consumption of vegetables and olive oil reported in the Arab population. In contrast, a high percentage of the Jewish population consumes a "Western-type" high fat/low fiber diet which may be associated with increased colon cancer risk (16-18). Separately or in combination, in addition to different genetic backgrounds, these factors may contribute to the differences in nationwide prevalence and presentation of CRC.

A higher percentage of Arab patients in our study presented with advanced stages of the disease and had cancers with poorly-differentiated or mucinous histology. Similar to the results of our study, recent data from a small case-control study on Palestinian patients with CRC showed a higher percentage of Palestinians with advanced stage CRC as compared to Jewish patients (19). Interestingly, these data differ from that published by Fireman et al., who found a similar distribution of Dukes' stage in both ethnic populations, although the number of Arab patients in their study was substantially lower than ours (12). Since the public medical services, the dominant health care providers in Israel, are equally available to the entire population of the country, it is unlikely that the higher percentage of delayed diagnosis in Arab patients with advanced disease was due to low availability of community health service. However, a lower awareness of the sector to early screening and to the early symptoms of the disease may be among the possible reasons for the delay in diagnosis of CRC, with the

## detected presentation pattern.

The younger age at presentation and higher percentage of mucinous and poorly-differentiated tumors found among Arab patients in our study might indicate that at least some of these cases belong to one of the hereditary non-polyposis colon cancer syndromes. In fact, our data indicate that $15 \%$ of Arab patients were under age 50 , and $21 \%$ had mucinous and poorly-differentiated tumors as compared to a much lower percentage found among Jewish patients. Studies from Egypt demonstrated a high percentage of MSI-positive poorly-differentiated and mucinous tumors among young Arab patients with CRC (20-22). Surprisingly, despite a higher percentage of patients with pathological features attributed to replication error-positive colon cancers, we found that loss of MMR proteins in the Arab patients was not statistically different from that of the Jewish patients who had fewer mucinous tumors and a significantly lower percentage of patients under 50 years old. Because of the retrospective nature of our study and the lack of detailed family history, we were unable to detect the patients with a hereditary CRC predisposition based on family history or revised Amsterdam criteria. There is a paucity of data on the frequency of MSI in Israeli Arab and Jewish patients with CRC. To the best of our knowledge, no population-based studies have been conducted in Israel. A recent study on a small selected group of 13 Arab patients' families who fulfilled loose Bethesda criteria and were below age 60 showed that $43 \%$ of tumors tested were MSI-H, although these data were not compared to a similar group of Jewish patients (23).

In summary, our data demonstrated that CRC patients from two major ethnic populations in Israel, Arabs and Jews, differ in terms of the prevalence of the disease, pathological features, age and stage at presentation, but not in the frequency of MMR-positive cancers. Despite being a potentially avoidable cancer in at least one-third of cases through early detection and treatment of precancerous lesions, CRC in the Israeli Arab population, although relatively rare, still represents a substantial health problem as it affects a young population and is found in advanced stages in a high proportion of patients. Although our retrospective study enrolled a limited number of Arab patients, it is, to date, the largest study conducted on this particular topic. Our findings urge the undertaking of larger population-based multicenter studies in order to understand substantial differences in CRC presentation and prevalence among different ethnic populations in Israel.

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