Hyperplastic polyps in hereditary nonpolyposis colorectal cancer

F E M Rijcken¹, T van der Sluis², H Hollema², J H Kleibeuker¹

Department of Gastroenterology¹ and Pathology², University Medical Center Groningen, The Netherlands.

Abstract

**Objective** - Hereditary nonpolyposis colorectal cancer (HNPCC) is a genetic syndrome caused by germline mutations in DNA mismatch repair (MMR) genes, in particular *hMLH1*, *hMSH2* and *hMSH6*. Dysfunction of MMR genes leads to loss of MMR protein expression and to microsatellite instability (MSI). MSI is also detected in 10-20% of sporadic colorectal cancers. Hyperplastic polyps (HP) may serve as precursor for these MSI+ sporadic colorectal cancers. The aim of this study was to examine whether hyperplastic polyps are also possible premalignant lesions in HNPCC.

**Methods** - All hyperplastic polyps resected from (suspected) mismatch repair gene mutation carriers were retrieved from a screening program database. Clinical information on the age at colonoscopy and the location of the HP was collected. MLH1, MSH2, and MLH6 protein expression was evaluated using immunohistochemistry.

**Results** - 90 HPs were resected from 21 male and 19 female subjects. The mean age at resection was 45.7 years (44.7 years in male and 46.6 years in female patients). Nineteen (21%) HPs were resected from the proximal colon, 23 (26%) from the distal colon, and 48 (53%) from the rectum. None of the hyperplastic polyps demonstrated loss of MMR protein expression.

**Conclusion** - Mismatch repair dysfunction in hyperplastic polyps of HNPCC patients is apparently very rare. It seems unlikely that hyperplastic polyps in HNPCC patients are precursors for (MSI+) cancers in these patients.
There is abundant evidence supporting the classical adenoma-carcinoma sequence in the evolution of colorectal cancer.\textsuperscript{1,2} Hyperplastic polyps (HPs) have not been included in the classical model. Traditionally, hyperplastic polyps have been regarded as benign lesions, lacking the potential for neoplastic progression. Recently, this view has become increasingly difficult to sustain given the finding of K-ras mutations\textsuperscript{3}, TGF-\textbeta\textsubscript{II} receptor mutations\textsuperscript{4}, chromosome 1p deletions\textsuperscript{5} and DNA microsatellite instability (MSI)\textsuperscript{6} in hyperplastic polyps.

With the discovery of the mismatch repair (MMR) genes it has become clear that two major molecular genetic pathways could be discerned in colorectal carcinogenesis. The first pathway, the chromosomal instability pathway, is characterized by allelic losses and gains and aneuploidy. The second pathway, the microsatellite instability pathway, is characterized by an abundance of subtle DNA mutations and diploidy. Microsatellite instability is caused by inactivation of a DNA mismatch repair gene. It is the molecular hallmark of hereditary nonpolyposis colorectal cancer (HNPCC), a genetic syndrome caused by germline mutations in DNA mismatch repair genes, in particular hMLH1, hMSH2 and hMSH6. Microsatellite instability seems to be an early event in the carcinogenesis of HNPCC colorectal cancers as up to 57\% of colorectal adenomas from patients with HNPCC show MSI.\textsuperscript{7,8}

Microsatellite instability is also detected in 10-20\% of sporadic colorectal cancers.\textsuperscript{9,10} However, on the basis of morphological observations and epigenetic changes these lesions are thought to progress to cancer in a way that differs from the microsatellite instability pathway described for hereditary MSI\textsuperscript{+} tumors. Jass et al. proposed the serrated pathway in which hyperplastic polyps serve as precursor for sporadic MSI\textsuperscript{+} colorectal cancers.\textsuperscript{6} Under the influence of promoter methylation of the DNA mismatch repair gene hMLH1 hyperplastic polyps may develop through serrated dysplasia into sporadic MSI\textsuperscript{+} colorectal cancer.

In HNPCC all polyps, even minute ones, are resected during screening colonoscopies. Part of these is hyperplastic and so far, these were considered to be innocent bystanders without any consequences for further policy. However, in view of the recently described microsatellite instability and loss of expression of mismatch repair proteins in hyperplastic aberrant crypt foci (ACF) from MMR gene mutation carriers the innocence of hyperplastic lesions in HNPCC should be questioned.\textsuperscript{11} On the other hand, Iino et al. found only two of 17
hyperplastic polyps in subjects with HNPCC to be microsatellite unstable.\textsuperscript{12} Jass \textit{et al.} described one HNPCC patient with a mixed hyperplastic polyp/adenoma in contiguity with a colorectal cancer.\textsuperscript{13}

The aim of this study was to examine whether hyperplastic polyps are possible premalignant lesions in HNPCC. The loss of expression of mismatch repair proteins is strongly associated with MMR gene dysfunction and, thus with MSI.\textsuperscript{14,15} Ninety hyperplastic polyps resected from (suspected-) MMR gene mutation carriers were evaluated for the presence of the mismatch repair proteins MLH1, MSH2 and MSH6 using immunohistochemistry.

**Materials and Methods**

In our hospital, all patients belonging to an HNPCC family are advised to be screened every two years by means of a colonoscopy. The clinical and the histopathological results are registered in a database. This database was used to retrieve all polyps, information concerning the age at the first and every consecutive colonoscopy, age at removal of a polyp, anatomical location of a polyp and final pathology report. The size of the hyperplastic polyps was determined by measuring the circumference of the polyp at 10x magnification. An experienced pathologist (HH) verified the histology.

In all hyperplastic polyps MLH1, MSH2, and MLH6 protein expression was evaluated using immunohistochemistry as previously described.\textsuperscript{16,17} Briefly, serial 3\textmu m-thick-sections were cut from paraffin blocks and fixed onto coated slides. Antigen retrieval was performed by high pressure cooker treatment of the slides in 200 \textmu l blocking reagent (Boehringer Mannheim, Germany). After blocking endogenous peroxidase activity, the primary antibodies diluted in phosphate buffered saline (PBS) with 1% bovine serum albumin (BSA) were applied for one hour. Slides were stained with MLH1 antibody (dilution: 1:500, clone G168-728, Pharmingen, San Diego, CA, USA), MSH2 antibody (1:100, clone Ab-2, Calbiochem, San Diego, CA, USA), and MSH6 antibody (1:200, Transduction, Lexington, KY, USA). After washing with PBS, the sections were incubated with rabbit antimouse peroxidase followed by goat antirabbit peroxidase, both diluted (1:50) in PBS-1% BSA. The peroxidase activity was visualized with diaminobenzidine. The sections were counterstained with haematoxylin.
To verify the correlation between proliferation and location of the mismatch repair protein immunostaining ten large hyperplastic polyps were assessed by immunohistochemical staining with a mouse monoclonal antibody to Ki-67 (MIB-1; 1:350, DAKO, Glostrup, Denmark).

Statistical analyses were performed using SPSS (Statistical Package for the Social Sciences, Munich, Germany) for Windows. The statistical difference between the location of hyperplastic polyps and adenomas was calculated using the nonparametric Mann-Whitney U test. P<0.05 was considered significant. The correlations between the presence of hyperplastic polyps and adenomas dependent and independent of the location were analyzed using Pearson's correlation coefficient.

Results

A total of 158 persons, 73 male and 85 female, were included in our screening program. Fifty (32%) were known mismatch repair gene mutation carriers. Twenty-two had a mutation in hMLH1, 21 in hMSH2 and 7 in hMSH6. The remaining 108 persons all belonged to families fulfilling the Amsterdam criteria.18 Five cancer patients (average age at diagnosis: 39 years) had uninformative genetic analysis. Eight genetic analyses were still in progress. Three of these eight persons had an HNPCC-related cancer (average age at diagnosis: 36 years), two had adenomas (average age at first resection: 31 years), while in the remaining three no neoplastic lesions had been identified (average age now: 41 years). In the remaining 95 persons genetic analysis had not been performed. Sixty-nine persons were from well-documented HNPPC families in which no germline mutation had been found up to this moment. Twenty-four of these persons had adenomas removed (average age at first resection: 44 years), in the remaining 45 no neoplastic lesions had been detected (average age now: 41 years). Genetic analysis had not been performed in another nine persons because their affected family members had yet to be tested. Another 17 persons were from HNPCC-families in which the mutation was known but the subjects chose not to be genetically tested. In this group two patients had one or more HNPCC-related tumors removed (average age at diagnosis of first cancer: 33 years), two women were diagnosed with atypical complex hyperplasia of the endometrium (average age at diagnosis: 43 years), five patients had
adenomas resected (average age: 44 years), and the remaining eight had no neoplastic lesions detected (average age now: 39 years).

The mean age at the first colonoscopy was 40 years. A total of 540 colonoscopies was done in 994 patient years. An average of 3 colonoscopies was done per patient. 257 colonoscopies in male patients rendered 41 hyperplastic polyps and 82 adenomas, while in female patients, 283 colonoscopies rendered 49 hyperplastic polyps and 75 adenomas.

Ninety hyperplastic polyps were removed from 40 patients, 21 men and 19 women. The mean age at which these hyperplastic polyps were removed was 46 years (45 years in male patients and 47 years in female patients). Nineteen (21%) hyperplastic polyps were resected from the proximal colon, 23 (26%) from the distal colon, and 48 (53%) from the rectum. The hyperplastic polyps size ranged from 1.0-14.0 mm with an average of 6.0 mm. The size of the hyperplastic polyps was not associated with the location in the colon (proximal: 6.2 mm, distal: 5.8 mm, rectal: 6.0 mm).

One hundred and fifty-seven adenomas were removed from 64 patients, 32 men and 32 women. The mean age at resection was 49.1 years. 82 (52%) adenomas were located in the proximal colon, 49 (31%) in the distal colon, and 26 (17%) in the rectum. Four adenomas with adenomatous as well as hyperplastic features from two MLH-1 mutation carriers were classified as serrated adenomas (figure 1). Two were located in the proximal colon, one in the distal colon and one in the rectum.

Figure 1. Serrated adenoma from a MLH1 mutation carrier.
In 18 colonoscopies synchronously occurring hyperplastic polyps (n=25) and adenomas (n=28) were resected. Three (12%) of these HPs were located in the ascending colon, 7 (28%) in the descending colon and sigmoid and 15 (60%) in the rectum while 19 (68%) adenomas were located in de ascending colon, 4 (14%) in the descending colon and sigmoid and 5 (18%) in the rectum (figure 2). In 387 colonoscopies neither hyperplastic polyps nor adenomas were observed. In 45 colonoscopies only hyperplastic polyps were resected and in 88 colonoscopies only adenomas. In 2 colonoscopies a cancer was observed. There was no correlation between the presence of hyperplastic polyps and adenomas or between the location of the two types of polyps.

Figure 2. Distribution of hyperplastic polyps and adenomas in the colon.

Hyperplastic polyp (●), synchronously occurring hyperplastic polyp (HP) with another HP (●), adenomas (X), synchronously occurring adenomas with another adenoma (x), number of colonoscopies (*). A, B, and C represent colonoscopies in which synchronously occurring hyperplastic polyps and adenomas were resected. If more than one hyperplastic polyp/adenoma were removed than the most distal hyperplastic polyp/adenoma was used as index polyp and marked with a large symbol while the synchronously occurring hyperplastic polyp(s)/adenoma(s) were marked with a smaller symbol. A, colonoscopies with proximal HPs with synchronously occurring adenomas. B, distal hyperplastic polyps with synchronous adenomas. C, rectal HPs with synchronous adenomas. D, colonoscopies in which only hyperplastic polyps were resected. E, colonoscopies in which only adenomas were resected. F, colonoscopies without polyps.
Figure 3. Hyperplastic polyp from a MSH2 mutation carrier demonstrating similar immunostaining pattern for (A) MSH2, (B) detail of MSH2, and (C) MIB, (D) detail of MIB.
Twenty-five (28%) of the 90 hyperplastic polyps were resected from 14 patients with a known mutation in one of the mismatch repair genes. Nine HPs were from 4 hMLH1 mutation carriers, 12 HPs were from 6 hMSH2 mutation carriers and 4 HPs were from 4 hMSH6 mutation carriers. The average age at resection was 51.2 years. Three (12%) hyperplastic polyps of known mutations carriers were found in the proximal colon, seven (28%) in the distal colon and 15 (60%) in the rectum.

None of the hyperplastic polyps or the serrated adenomas showed loss of expression of one of the mismatch repair proteins. The immunohistochemical staining in the crypts of the hyperplastic polyps was similar to that in crypts of normal epithelium of the colorectum. Most nuclei in the base of the crypts expressed all three MMR proteins intensely while few to none of the luminal nuclei expressed any of the MMR proteins (figure 3 A and B). Expression of mismatch repair proteins overlapped with proliferation areas as demonstrated by Ki-67 immunoreactivity (figure 3 C and D).

Discussion

Hyperplastic polyps are possibly precursors of sporadically occurring microsatellite unstable tumors. Even though hyperplastic polyps are common and hyperplasia may occur in contiguity with adenomatous lesions in HNPCC, the present study illustrates that hyperplastic polyps most likely do not play a significant role in the carcinogenesis of microsatellite unstable tumors in subjects with a germline MMR gene mutation.

During an average follow-up of 6 years, 25% of HNPCC patients had hyperplastic polyps removed. Eight percent of the patients had hyperplastic polyps removed at their first colonoscopy. In a study by Imperiale et al, 10% of asymptomatic average risk persons 40 to 49 years of age had hyperplastic polyps. Hyperplastic polyp prevalence increases with age and has been reported to be up to 35%. In the present study, 185 of the 540 colonoscopies were performed in patients older than 50 years. The majority (79%) of hyperplastic polyps of HNPCC patients were located in the distal colon and rectum as is seen in sporadic cases. In contrast to sporadic colorectal cancer, hereditary nonpolyposis colorectal cancer has a predisposition for the proximal colon. We have shown in the past that adenomas in HNPCC patients also have a predisposition for the proximal colon and that these proximal adenomas
are highly dysplastic at a smaller size than sporadic adenomas. In the present study, the presence or location of hyperplastic polyps did not correlate with presence or location of adenomas. These characteristics are not in line with the theory that hyperplastic polyps play a role in HNPCC carcinogenesis. On the other hand, the detection of four serrated adenomas could support the premalignant theory of hyperplasia. However, neither the hyperplastic nor the adenomatous part of the serrated adenomas demonstrated loss of one of the mismatch repair proteins.

None of the hyperplastic polyps included in this study showed loss of expression of MLH1, MSH2 or MSH6. Only two other studies have been published concerning hyperplasia in colorectal lesions of HNPCC patients. In one study, microsatellite instability was reported to be present in hyperplastic aberrant crypt foci occurring synchronously with MSI+ colorectal cancer. In the other study, 2 (11%) of 17 hyperplastic polyps were found to have a low degree of microsatellite instability (MSI-low). One case report describes a mixed hyperplastic polyp/adenoma in contiguity with a colorectal cancer in an HNPCC patient. Compared to the above studies, our results seem to underestimate the incidence of mismatch repair dysfunction in hyperplastic polyps in HNPCC patients. However, the studies are not readily comparable due to the different research populations (HNPCC patients with MSI-high colorectal tumors vs. an HNPCC screening population) and the different techniques used (microsatellite analysis vs. immunohistochemistry). Pedroni et al and Jass et al both detected a low degree of microsatellite instability and not a high degree (MSI-high) in the hyperplastic polyps. Immunohistochemistry most often cannot discern MSI-low hyperplastic polyps as MSI-low lesions generally do express mismatch repair proteins. Whether this should be considered a limitation of our study is debatable. The significance of MSI-low is controversial. MSI-low lesions have not been consistently shown to be different from microsatellite stable lesions. HNPCC lesions are most often associated with the MSI-high phenotype which is undoubtedly different from the microsatellite stable phenotype.

Genetic inactivation of a mismatch repair gene is generally associated with loss of immunohistochemical expression of the corresponding protein. Lindor et al demonstrated that immunohistochemistry had a sensitivity of 92.3% and a specificity of 100% for screening for DNA mismatch repair defects. Other recent studies have concluded that immunohistochemical analysis of MLH1 and MSH2 expression is a rapid and accurate
method for identifying colorectal tumors of the MSI-high phenotype.\textsuperscript{25-28} We also confirmed the high quality of mismatch repair protein immunohistochemistry in (pre)malignant lesions in comparison with microsatellite instability analysis.\textsuperscript{29}

MMR deficiency results in microsatellite instability and it is thought to cause cancer by failing to repair replication errors within repeat sequences contained in genes relevant for growth control and differentiation like TGF-\(\beta\)RII, BAX, and IGFRII. Due to the MMR deficiency, mutation rates in tumor cells are 100-1000-fold as compared to normal cells.\textsuperscript{30,31} The high mutation rate leads to an accelerated adenoma-carcinoma sequence in HNPCC.\textsuperscript{2} Pedroni et al demonstrated progressive accumulation of bandshifts in microsatellite analysis in the sequence normal mucosa-dysplastic aberrant crypt foci-adenoma-carcinoma indicative of the role of MMR gene dysfunction in the evolution of colonic lesions.\textsuperscript{11,32,33} Iino proposed that MSI in hyperplastic sporadic polyps heralds the transformation to serrated adenoma.\textsuperscript{6} Similarly, we demonstrated that loss of expression of MMR protein heralds development to high-grade dysplasia in HNPCC adenomas.\textsuperscript{16}

In conclusion, this is the largest series of hyperplastic polyps from subjects with HNPCC or at 50\% risk for it, which were evaluated for mismatch repair dysfunction. None of these hyperplastic polyps nor any of four serrated adenomas displayed loss of expression of the three MMR proteins. If hyperplastic lesions would play a significant role in the microsatellite instability carcinogenesis pathway of HNPCC lesions loss of expression of MMR proteins should have been detected at least in some of the polyps. Our findings do not dictate any changes in clinical practice. Mismatch repair dysfunction in hyperplastic polyps of HNPCC patients is apparently very rare and if found it should be considered a coincidence and not a common step in the carcinogenic pathway of HNPCC.
References


