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Colorectal Cancer: Epidemiological Study, Clinical, Histological and Immunohistochemistry Examination in Patient of West Algeria

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Abstract

Colorectal cancer (CRC) is significant publichealth problem in rich countries of the world, because of its frequency and mostly because of its severity. Our aim was to improve histologic diagnosis of malignancy with topographic coloration (HE) and cytokeratins 7, 20 immuno-labeling. The risk for colorectal cancer incease with age, but in our series, 10% of colorectal cancer exist in patients younger than 40 years old. In histological study, adenocarcinoma represented 100% and the evaluation for stage of disease showed that the most dominant isstage IV (32.6%). In our trial, we found that cytokeratins 7 and 20 immunoreactivity in colorectal adenocarcinoma are often positive for cytokeratin-20 and most of time negative for cytokeratin-7.

Keywords

Adenocarcinoma, Colorectal Cancer (CRC), Cytokeratin (7, 20)

1. Introduction

Worldwide, the number of new cases of colorectal cancer was estimated at 1.4 million in 2012. This shows a relative increase in the incidence of these cancers. For this period by its frequency,CRC arranged third in men with 746,000 new cases and second in women with 614,000 new cases [1].

In Algeria, the incidence of colorectal cancer (3380 cases/year, or 8.9%) is in second place after breast cancer (8177 cases/year 21.6%). It is the second cancer in humans (1690 cases or 10.3%) after lung cancer [2].

Five-year survival rate in colorectal cancer is about 60% - 95% in the initial stages and decreases dramatically to 35% in stages where lymph node metastases are detected [3]. These statistics are baleful because cancer is a well-studied malignity, which has a slow progression, known risk factors and pre-neoplastic lesions that can be detected

and treated [4]. Because the warning symptoms and signs are belated, colorectal neoplasm is found most often in the later stages, which drastically reduces the chances of applying radical curative treatment. Hence, the need is to introduce measures for colorectal cancer screening [5] and a better management of patients with this type of neoplasia. The aim of this study is to evaluate histological and immunohistochemical status of patients with metastatic colorectal cancer.

2. Patients and Methods

The statstical test included 166 colorectal cancer patients hospitalized in the Medical Oncologie Service in Oran University Hospital Center (CHUO), between January 2013 and Febrary 2016, aged between 25 and 80 years.

The data were recorded in clinical observation sheet of each patient.Clinical and laboratory data were entered into a Microsoft Excel database for statistical processing. With the program functions or averaged variables, confidence intervals, standard significance tests were performed comparing the data series. The statistical significance of the values was considered as $p < 0.05$.

For the histopathology study, there were surgically taken fragments of tumor tissue, which were fixed in 10% buffered formalin for 48 - 72 hours and processed for histological techniques including the classic paraffin. Paraffin-included samples were cut using the microtome in 4-µm sections and then stained with Hematoxylin-Eosin. Immunohistochemical study aimed to label abnormal intestinal epithelium cells. For immunohistochemical study of paraffin-included material, we selected a total of 30 cases of colorectal adenocarcinomas. Since the histological sections were made of 3-µm thickness, biological material was collected on slides superfrost ultra plus, after which they were kept in thermostat at 37˚C for 24 hours to increase the adhesion of biological material. Following deparaffination and hydration of the histological sections, We pre-heat steamer or water bath with staining dish containing Sodium Citrate Buffer or Citrate Buffer until temperature reaches 95˚C - 100˚C, immerse slides in the staining dish. Place the lid loosely on the staining dish and incubate for 20 - 40 minutes (optimal incubation time should be determined by user). Washed in tap water before being cooked in citrate pH 6 solution for 20 minutes to expose the antigen. After boiling, the solution was washed using phosphate-buffered saline (PBS).The sections were incubated with primary antibodies overnight, at 4˚C, and the next day the signal was amplified for 30 minutes using the secondary antibody immpact DAB peroxidase substrate (EnVision, Dako). The signal was detected with 3,3'-Diaminobenzidine (DAB, Dako). For immunohistochemical study, we used the antibodies found in Table 1 below.

Table 1. Antibodies used in the study.

Antibody	Producer	Clone	Clonality	Unmasking	Dilution
Cytokeratin7	Dako	OV-TL 12/30	Ms Monoclonal	Sodium citrate, pH 6	1:200
Cytokeratin20	Dako	Ks20.8	Ms Monoclonal	Sodium citrate, pH 6	1:200

Ms—mouse.

3. Results

In our series, the risck of most of types of malignancies in colorectal cancer increases by age. The youngest patient in the group was 23 years (diagnosed withrectal villous tumor), and the oldest was 81 years (diagnosed with lower rectal adénocarcinoma) (Figure 1 & Figure 2).

Concerning the evolutionary stage of studied colorectal cancers, clinical, laboratory and intraoperative data allowed us to note that out of the 166 tumors, 12 (7.41%) have been diagnosed in stage I, 47 (28.14%) in stage II to stage III, 53 (31.85%), and 54 (32.6%) in stage IV (Table 2).

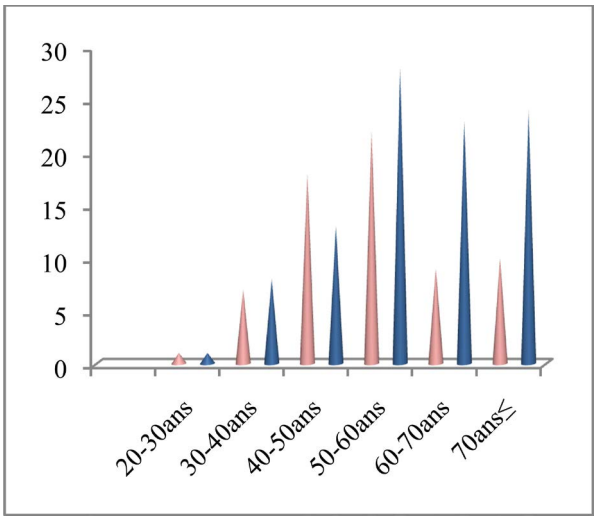

Figure 1. Distribution of patients by age.

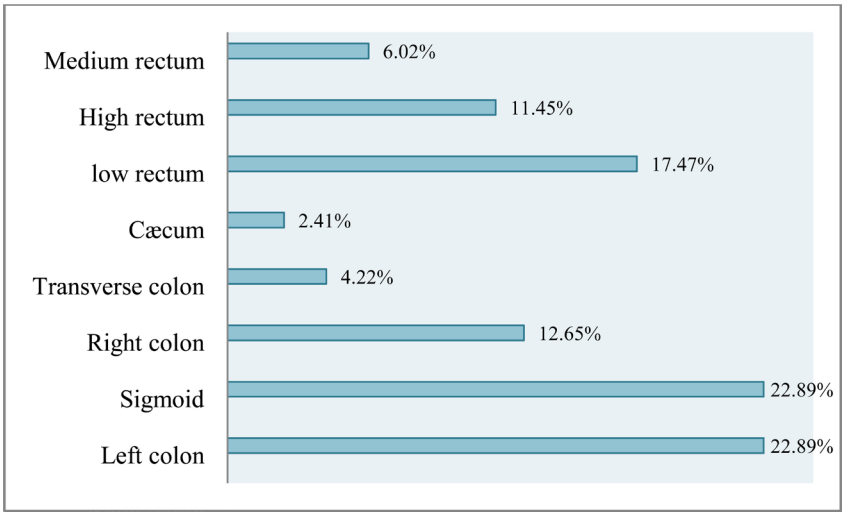

Figure 2. Topographic distribution of the studied group.

Table 2. Evolutionarystage of the colorectal adenocarcinome studied group.

Stages	I	II	III	IV
Patient Number	12	47	53	54
(%)	7.41	28.14	31.85	32.6

Histopathological study showed that out of 166 cases of colorectal cancer, 166 (100%) cases were adenocarcinomas. Regarding the adenocarcinomas, 143 (86.4%) cases were Lieberkuhnian adenocarcinomas, 20 (12.24%) cases were diagnosed as mucinous adenocarcinomas and 3 (1.36%) patients were represented by the "signet ring cell" carcinomas. Regarding the degree of cell differentiation, out of the 143 adenocarcinomas, a number of 110 (66.43%) were well-differentiated adenocarcinomas, 28 (16.43%) were moderately differentiated, and 5 (2.86%) cases were poorly differentiated adenocarcinomas.

For histopathological and immunohistochemical study we have choose30 specimens from the archives of the Privat Laboratory (A. KORSO) of Pathology of pure adenocarcinomas of the colon processed to paraffin from as many patients, that were operated in the General Surgery (CHU and EHU). we selected cases of pure adenocarcinoma because they represented more than 3/4 of operated colorectal tumors. Histopathological and immunohistochemical aspects varied greatly from one tumor to another. In all tumor types, concerning of the degree of differentiation we have observed many mitoses, including atypical mitotic figures.

The well-differentiated adenocarcinomas were all shown to contain immature mucous cell in varying proportions and cells with abundant microvilli (Figure 3(a) and Figure 3(b)). In moderately differentiated adenocarcinoma, we observed unequivocal intracytoplasmic mucin vacuoles, cellular and nuclear atypia were more numerous (Figure 4). The most extensive cellular changes occurred in poorly differentiated adenocarcinomas, generally composed of sheets of undifferentiated cells (Figure 5).

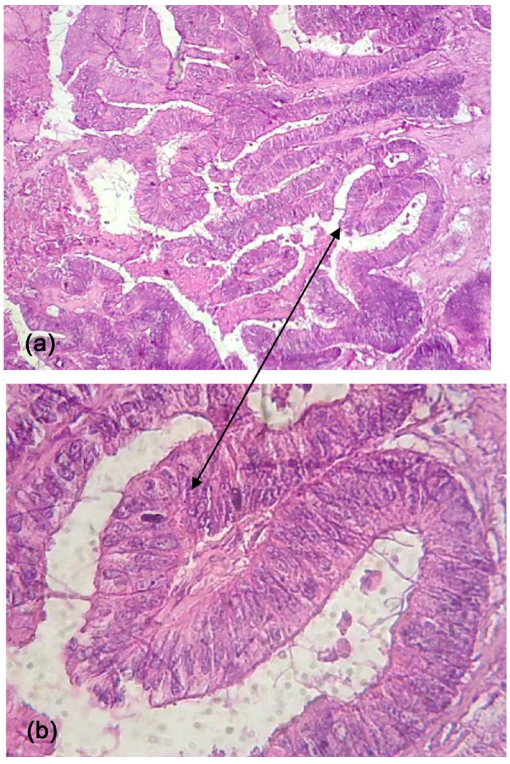

Figure 3. (a) Colon adenocarcinoma infiltrating in the mucous tunic. Hematoxylin-Eosin staining, 10×; (b) Well-differentiated adenocarcinoma with polymorphic epithelium, partially pseudostratified, which delineates large, irregular lumen glands filled with tumor, inflammatory or partially degraded cells. Hematoxylin-Eosin staining, 40×.

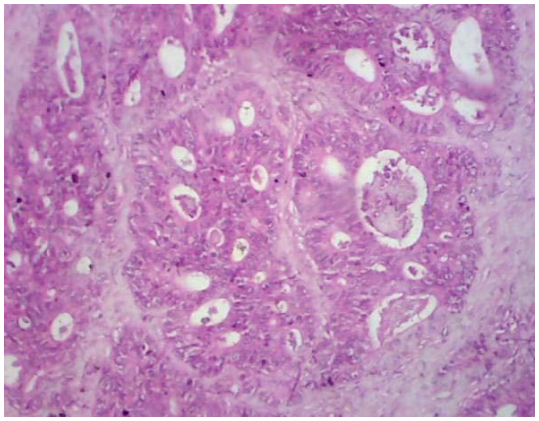

Figure 4. Image of moderately differentiated colon adenocarcinoma. Hematoxylin-Eosin staining, 10×.

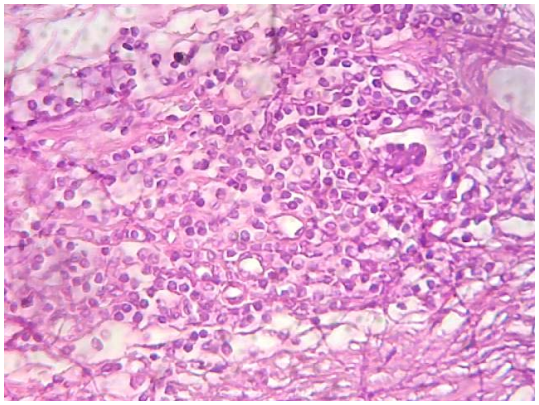

Figure 5. Image of poorly differentiated adenocarcinoma associated with moderate inflammatory reaction, 40×.

Interpretation of immunohistochemical reactions focused primarily over highlighting the chromogen on the antigenic targets and the number of positive tumor cells. Thus, if no cell has been immunohistochemically labeled, we considered an absent reaction; if the reaction was positive in less than 10% of examined cells with a microscope objective of 20×, we considered a poor response; if they were positive in 10% to 25% of the cells, we considered a moderate reaction reaction, and if it was positive in more than 25%, we have found that the reaction was intense.

Several panels of immunohistochemical markers have been developed to allow for recognition of patterns suggestive of specific tumor types. One such immunohistochemical phenotype charachterized by markers commonly seen in colon cancer is inceasingly being recognized as a distinct favorable subset of cancer of unknown primary (CUP). We investigated the expressions of CK7 and CK20 in 30 cases of colorectal carcinoma [6] (**Figures 6-10**).

4. Discussion

The colorectal cancer deaths rates have dropped significantly in the past couple of decades. The death rate (the number of deaths per 100,000 people per year) of colorectal cancer isdecreasing,presumably through increased colorectal cancer screening [7]. In UK

theincidence by age stratification is 4 cases/100,000 for people under the age of 50, 100 cases/100,000 for those aged 50 - 69 and 300 cases/100,000 for those over the age of 70, same result are obtained in our study where the incidence rate of CRC has increased solely in people over 50 years, something [8], overall it growth dramatically after 40 years in patients in urban areas and those in rural areas [9]. which suggests that colorectal cancer typically results from a complex interaction between genetic and environmental influences.

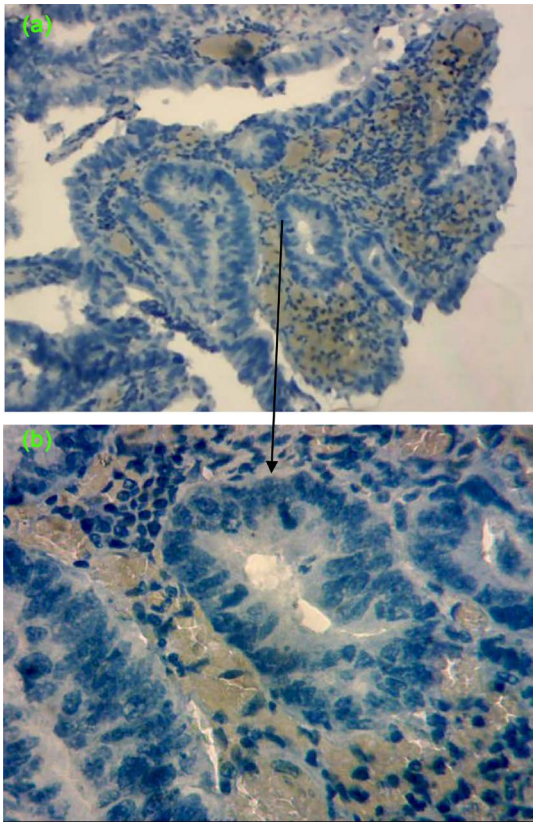

Figure 6. Well-differentiated adenocarcinoma with intense reaction to CK20. Anti-CK20 antibody immunostaining (a) 10×, (b) 40×.

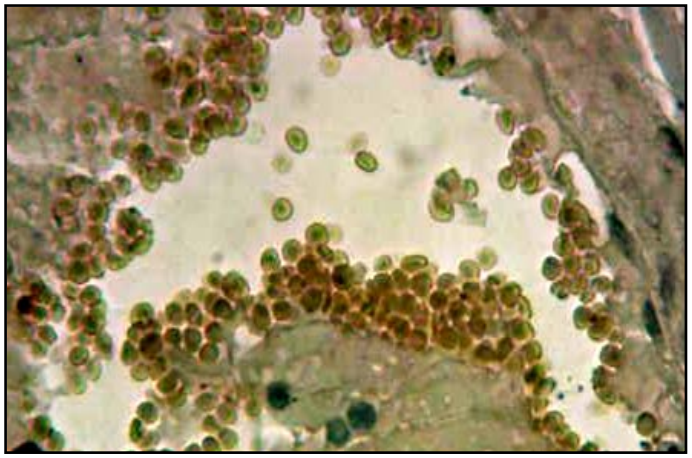

Figure 7. Poorly differentiated adenocarcinoma with moderate reaction to CK20. Anti-CK20 antibody immunostaining, 40×.

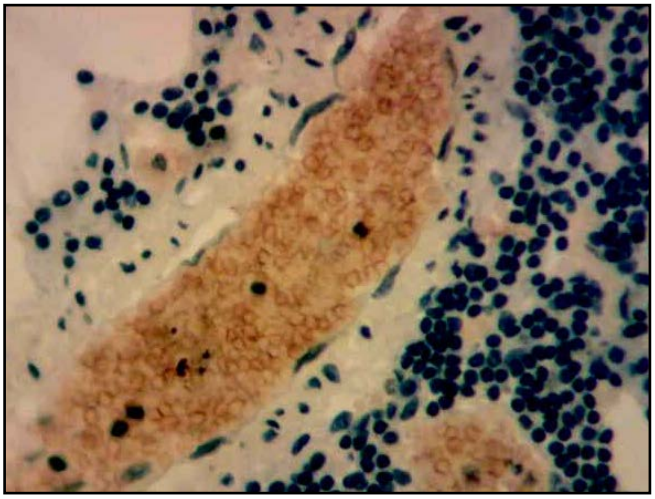

Figure 8. Mucinous adenocarcinoma with moderate reaction to CK20. Anti-CK20 antibody immunostaining, 40×.

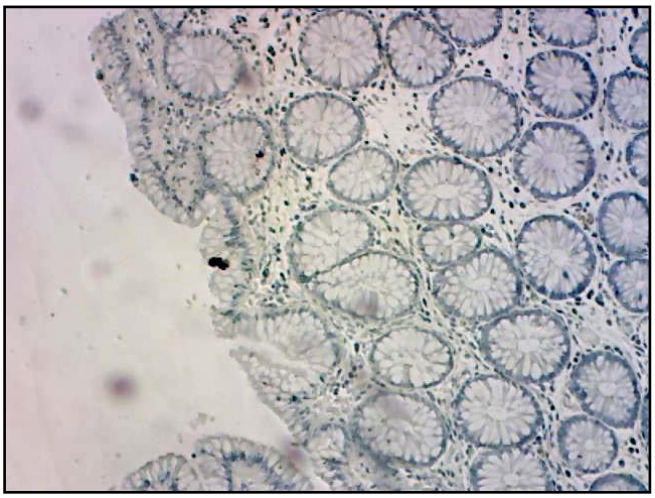

Figure 9. Normalcolonic mucosa showed negative staining to CK7. AntiCK7 immunostaining 100×.

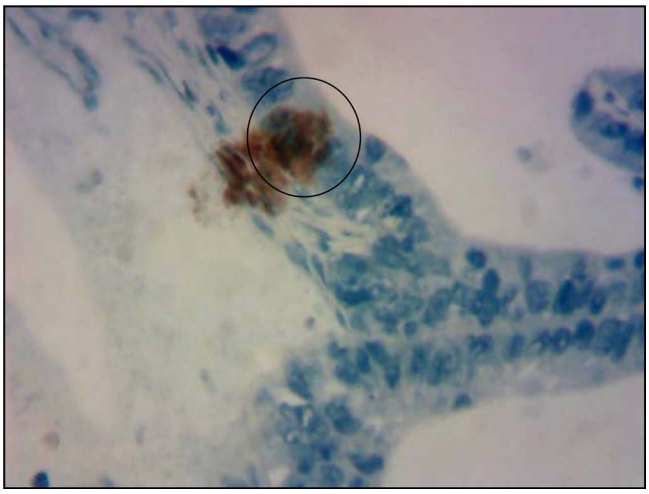

Figure 10. Well-differentiated adenocarcinoma with Weak reaction to CK7. Anti-CK7 antibody immunostaining 40×.

Investigations have shown that DNA damage and somatic mutations occur during cancer progression in humans. [10]. In humans mucus production and composition changes with age [11]. All these data highlights the complex nature of malignant degeneration in the colon and the importance of age as a risk factor in the development of this cancer.

The difference in sex of patients with colorectal cancer, was not statistically significant. Of 166 patients with colorectal cancer, 68 (41%) were females and 98 (59%) males, According to some scientists the difference of incidence of such disease has to do with gender [12] [13] [14] [15].

In our study, tumor localization was predominant in the colon if we compare it with the segments of rectum. Two-third of colorectal cancer occur in the left colon and one-third in right colon, although women more often develop right-sided tumors. About of 20% of colorectal cancers develop in the rectum [16].

Regarding the tumor stage, we found that over 60% of patient in our series were diagnosed with stage III and IV tumors. 21% of colorectal cancers are diagnosed late, but behind time diagnosis is more common among some sociodemographic groups than others.It is distinctly clear that people living in areas with high proportion of poverty and unemployment have their colorectal cancer diagnosed at a late stage by comparing them with those living in other areas. Other authors have reported similar dataIn a study in Tunisia,analyzing a number of 280 colorectal cancers diagnosed, found that 25% were found to have stage I/II disease, 52.5% stage III and 22.5% with stage IV cancer [17].

Tumor grade refers to themicroscopic appearence of a malignancy of a predetermined class and histologic type and the mesure to wich it resembles the tissue of origin. In our series, more than halfof the tumors were classified as well-differentiated adenocarcinoma.

By against to some authors, moderately differentiated adenocarcinoma of the colon and rectum cancers can reach up to 70% [18].

The identification of the metastatic carcinoma of unrevealedsource can be very difficult. The determination of the primary site of the metastasis is a challenge to one and the other oncologists and pathologists, having conceivably important clinical and therapeutic consequences [19] [20]. Analysis of the expression pattern of CK7 and CK20 can give the clinician a suggestion of the origin of the tumor [11] [21].

Differential cytokeratin expression may be of use with intestinal tumours showing a different profile (Cytokeratin 20 positive/7 negative).

Normal epithelium of the small bowel, appendix and colorectum, and adenocarcinomas from these sites, are almost consistently CK7−/CK20+, helping to distinguish these adenocarcinomas from adenocarcinomas of many other primary sites [22] [23].

The CK7−/CK20+ pattern was identified in 65% to 95% of the colorectal adenocarcinomas in different series [9] [24] [25] [26] [27]. The CK7−/CK20+ immunopheno type was expressed by 20 (66.66%) colorectal tumors, CK7+/CK20− 7 (23.33%)and CK7+/CK20+ 3 (10%) in the present study. Therefore by colonic epithelial tumors. Interestingly, several reports have described CK7 expression in colorectal adenocarcinoma, with expression ranging from 5% to 74% [9] [24] [25] [26] [27].

CK20 reactivity was diffuse (more than 50% of cells were positive) in our cases and in

the majority of colorectal carcinoma cases and mainly focal (<50% ofcells were positive) in gastric and pancreatic adenocarcinomas as in previous studies [25] [26] [27] [28].

5. Conclusion

In conclusion, colorectal cancer is affecting together women and men in relatively equal proportions. Most cancers were recorded in the left colon. More than half of the patients were staged III and IV at first admission. The histopathological study showed that about 100% of colorectal neoplasms were adenocarcinomas, frequently with well differentiation. Immunohistochemistry for CK7 and CK 20 help differentiate primary colorectal adénocarcinoma from metastasis. In our study, we found that more than 20% of patients with ck20+/ck7− phenotype had hepatic metastasis.

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