Prognostic values of detecting MSI phenotypes in colorectal carcinoma by immunohistochemicalmethod compared to molecular investigation

Etude comparative de la valeur pronostique dans la détection des phénotypes MSI dans le carcinome colorectal par immunohistochimie en comparaison avec l'analyse moléculaire

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RÉSUMÉ

Introduction: L'identification essentiellement des altérations de MSH2et/ou de MLH1a une implication clinique pour la reconnaissance et le pronostic des phénotypes MSI. Dans cette étude, nous avons évalué l'instabilité par immunohistochimie et confirmer les résultats par des analyses moléculaires et ainsi de démontrer en routine son utilité comme un facteur prédictif dans la détermination de l'instabilité micro-satellitaire chez des patients atteints de carcinome colorectal

Méthodes : Quarante-sept tissus tumoraux et leurs muqueuses coliques saines adjacentes ont été sélectionnés rétrospectivement pour cette étude .Nous avons en premier lieu étudié la valeur potentielle de l'analyse moléculaire pour l'identification de l'instabilité micro- satellitaire dans laquelle un panel de 5 marqueurs microsatellites a été choisi (Bat-25,Bat-26, D2S123, D5S346 and D17S250). En second lieu, nous avons évalué le profil immuno histochimique des protéines MMR à savoir MLh1,MSH2, MSH6 et PMS2 dans le tissu tumoral en comparaison avec le tissu sain adjacent.

Résultats: 14 cas ont été considérés comme étant instables et les cas restants sont considérés comme stables. De plus, nous avons trouvé une perte d'expression de MLH1, MSH2, MSH6 et PMS2 dans 9, 10, 6 et 9 cas, respectivement. Les patients avec un statut MSI étaient âgés moins de 45ans, avaient une localisation droite et de type histologique mucineux. Nous avons trouvé une association entre MSh2, l'âge (p=0.03) et le stade TNM(p=0.02).

Conclusion: nous avons trouvé une association entre l'investigation moléculaire et l'expression immuno histochimique des protéines MMR ce qui permettra d'identifier spécifiquement ces phénotypes MSI chez des patients atteints de cancer colorectal dont le but est de leur éviter une chimiothérapie à base de 5FU volontiers inefficace. Cependant, l'analyse immuno histochimique des protéines MMR pourrait être utilisée en routine pour la détection de l'instabilité sans vraiment avoir recours à l'analyse moléculaire.

Mots-clés

Instabilité micro-satellitaire, cancer colorectal, immunohistochimie, protéines MMR, investigation moléculaire, panel de Bethesda

SUMMARY

Background: The identification essentially of hMSH2 and/or hMLH1 alterations has clinical implications for recognition and prognosis of MSI phenotypes cases. In this study, we tried to identify instability by immunohistochemical expression pattern analysis, compared the results with molecular investigation and shown their usefulness as predictive factors for determination of Microsatellite Instability in patients with colorectal carcinomas in routinely.

Methods: Forty seven colorectal cancers and their adjacent colonic mucosa were selected retrospectively for this study. We first studied the potential value of molecular investigation to identify microsatellite instability in which a NCI panel (or Bethesda panel) of five microsatellite was analyzed (Bat-25, Bat-26, D2S123, D5S346 and D17S250). Secondary, we evaluated the immunohistochemical assessment of hMLH1, hMSH2, hMSH6 and PMS2 proteins in tumor and adjacent normal colorectal mucosa tissues.

Results: Fourteen cases were scored as MSI and the remaining MSS. Moreover, we found loss of expression for hMLH1, hMSH2, hMSH6 and PMS2 respectively in 9, 10, 6 and 9 of cases. The MSI patients were less than 45 years old, have right localization and mucinous histological type. We found an association between MSH2, age (P=0.03) and staging (P=0.02). MLH1 is associated only with age (P=0.02) while MSH6 with tumor grade (P=0.01).

Conclusions: We found an association between MSI molecular investigation and MMR immunohistochemical expression which may allow one to specifically identify MSI phenotype of patients with colorectal carcinomas. Furthermore, immunohistochemical analysis of MMR protein can be used in routinely for detection of microsatellite instability without occurs to molecular investigation.

Key-words

Microsatellite instability, colorectal cancer, immunohistochemistry, MMR protein, Bethesda panel.

Colorectal cancer (CRC) is one of the most common causes of cancer related death in the world [1, 2]. Its initiation and progression may involve different genetic pathways (Microsatellite Instability (MSI), Chromosomal Instability (CIN) and CpG Island Methylation Promoter (CIMP) by which tumor suppressor genes, oncogenes and Mismatch Repair genes (MMR) are altered and promote the tumor evolution [3, 4]. MSI phenotype is observed in approximately 15 to 25% of sporadic colorectal carcinoma and majority of HNPCC cases. This phenomenon is due to the inactivation of MMR genes, such as *hMSH2*, *hMSH6*, *hMLH1* and *hPMS2* [5] that results either from epigenetic alterations and/or mutations [4]. Indeed, inactivation of one of these genes caused microsatellite instability which characterized by alterations in the length of simple repetitive microsatellite sequences present in genome. Although, identification of this phenotype in colorectal carcinomas tends to have improved overall survival and better response to adjuvant treatment than microsatellite stable (MSS) tumors. The determination of instability by molecular biology is used routinely as a first-line for diagnosis of suspected HNPCC and for their clinical implication in sporadic colorectal cancer [5]. The “Bethesda criteria” also allow identification of HNPCC patients such as individual, familial and tumors characteristics and contrary to sporadic tumors, they have poorly differentiation and mucinous histology. So, MSI status is evaluated by the analysis of a reference panel (NCI panel) of five microsatellites using fluorescent polymerase chain reaction (PCR) followed by Fragment DNA analysis.These different markers are classified according to the level of their instability detected as High (MSI-H), Low (MSI-L) and Stable (MSS) [6]. MSI-H is usually caused by methylation of the promoter of *hMLH1*and/or *hMSH2*genes for sporadic tumors. Julian and *al.* (2001) reported that annual incidence of MSI-H tumors is about 20.000 to 26.000 each year in the United States. Indeed, these tumors have a better prognosis than MSI-L and MSS, independently of tumor stage cancers and they are associated with a lower risk of second offense and metastasis [7]. In addition, identification of alterations in MSI by molecular biology is expensive and time consuming and not majority of patients benefit before decisions of adjuvant treatment. Histopathological characteristics also play an important role for identification and recognition of patients with MSI status like poorly differentiation, right localization and mucinous type. On the other hand, Microsatellite Instability can be also detected by immunohistochemical(IHC) analyses, using antibodies against MMR proteins. The MSI phenotype is assigned in the majority cases the loss of expression of hMLH1 or/and hMSH2 in stoma [6]. The detection of those proteins by IHC can be helpful and fast in selection of CRC patients with MSI phenotype [5]. In this study, we tried to identify these phenotypes by immunohistochemical expression pattern analysis, compared the results with molecular investigation and shown their usefulness as predictive factors for determination of Microsatellite Instability in patients with colorectal carcinomas.Afterward, we correlated the IHC expression to clinical, pathological, surgical and therapeutic data.

METHODS

Tissue samples.

Resection specimens obtained retrospectively from 47 patients within period 2010 to 2011, who underwent surgery of CRCs at CharlesNicolle’s Hospital, Tunisia. All patients signed an informed consent, which permitted the use of their specimens for research. Detailed family histories were obtained through questionnaire with patients and their family. Only, the surgical and adjuvant treatment were retained. Biopsies and patients with Cohn’s syndrome were excluded. For these colorectal tumors, the following variables were assessed: tumor location (right, transversal and left colons, recto-sigmoid region and rectum), histological type (tumors in which less than 10% of the cells glands were classified as high grade, while those containing more 50% extracellular mucin were classified as mucinous type), lymph node metastasis (negative or positive), presence or absence of histo-prognostic criteria (peri-nervous engainement or vascular embolus). Tumors were classified in the regrouped tumor-node-metastasis (TNM) staging system from International Union against cancer (UICC).

DNA extraction.

Tissues from patients with CRCs were fixed in 10% formaldehyde solution, dehydrated and embedded in paraffin for histological and molecular analyzes. Two blocks contains each one tumor and adjacent colonic mucosas were selected for molecular instability analysis. Approximately, 3 to 6 sections of 5 µm were chooses for this study. Extraction was performed using the QIAamp DNA FFPE Tissue kit (Cat No.56404, Qiagen) according to the manufactory’s protocol.

Analysis of MSI.

DNA from tissues was amplified for the mono-nucleotide like Bat-25 and Bat-26 and the di-nucleotides markers such as D2S123, D5S346 and D17S250. Each pair of primer was labeled with the fluorescent markers FAM. These microsatellites were amplified in polymerase chain reaction (PCR) containing 1X KappaTaq Buffer, 2 mM $MgCl_2$, 0, 2mM each dNTP, and 1U Hot start KappaTaq DNA polymerase in a volume final equal to 25µl. the PCR was performed under the following conditions: denaturing at 95°C for 5 min, 35 cycles of denaturing at 95°C, annealing at 55 to 60°C for 30 seconds and extension at 72°C for 30 seconds. This was followed by an extension final at 72°C for 10 minutes. Products of PCR were

analyzed on 5% denaturing gels run in Applied Biosystems AB HITCHI 3500 automated DNA analyses. Gene Mapper® software (v5.0) was used to appreciate the size of each fluorescent PCR product. The profile (molecular weight) of each marker stemming from adjacent normal mucosa is stacked in that stemming from neoplasic colorectal mucosa. MSI-H was retained if at mean two of five markers analyzed were instable. MSI-L if one of all markers was instable. MSS if none of the five microsatellites analyzed was instable. A summary of the amplified markers and primer sequences is shown in Table 1.

Immunohisochemistry stain.

One block was selected for IHC. It comprised an area of normal colonic mucosa adjacent to the tumor. Three-micrometer-thick sections were prepared and mounted on silanated slides. The MMR antibodies were used for IHC analysis using the automated tissue staining system (VENTANA BENCH MARK GX) according to manufactory's protocols. All antibodies used in this study are listed in Table 2.In practice, slides were deparaffinized with toluene and hydrated with decreasing ethanol. Heat-induced antigen retrieval was performed using the VENTANA CCI mild reagent (VENTANA Medical systems).For blocking nonspecific protein binding, slides were treated with 10% normal goat serum. After, the Endogenous Peroxidase activity was blocked by incubation with 3% H_2O_2. Then, incubation of slides with pre-diluted primary antibodies against hMLH1, hMSH2, hMSH6 and PMS2, followed by incubation with Horseradish Peroxidase-conjugated antibody reagent. The antigen-antibody reaction was visualized using diaminobenzidine as a chromogen. Finally, the slides were lightly counterstained with hematoxylin. Loss of expression was estimated when nuclear staining was absent or less than 10% of stroma tumors cells, but preserved in normal epithelial. Lymphocytes staining were considered as positive control.

Statistical analysis.

For statistical analysis, the χ^2 was used to test the significance with regard to the immunostaining expression of MMR protein, MSI phenotype and clinico-pathological data. $P<0.05$ was considered as statistically significant and statistical results were obtained using EpI6 and Fisher test.

RESULTS

Clinical and pathological features.

Clinical and pathological characteristics of patients are summarized in Table 3. Eight (17.02%) probands were under 40 years of age; 33 (70.21%) aged more than 50 years, including 7 aged more than 80 years. Twenty-four

Tableau 1 : variation des sensibilités et spécificités dans différentes séries de la littérature **:** Characteristics of five nucleotide repeats used for MSI detection

Primer name	Microsatellites	Locus	Sequences	Length of Amplicates (pb)	T°C
1-F	Bat-25	1p31.1	TCG CCT CCA AGA ATG TAA GT	Around 125	54
1-R			TCT GCA TTT TAA CTA TGG CTC		
2-F	Bat-26	2p	TGA CTA CTT TTG ACT TCA GCC	Around 120	54
2-R			AAC CAT TCA ACA TTT TTA ACC C		
3-F	D2S123	2p16	AAA CAG GAT GCC TGC CTT TA	210-230	55
3-R			GGA CTT TCC ACC TAT GGG AC		
4-F	D5S346	5q21-22	ACT CAC TCT AGT GAT AAA TCG	110-130	57
4-R			AGC AGA TAA GAC AGT ATT ACT AGT T		
5-F	D17S250	17q11.2-q12	GGA AGA ATC AAA TAG ACA AT	150-160	58
5-R			GCT GGC CAT ATA TAT ATT TAA ACC		

Tableau 2 : variation des sensibilités et spécificités dans différentes séries de la littérature **:** Characteristics of Antibodies used for immunohistochemistry analysis

Antibodiesagainst	Source	Clone	Dilution	Type	Ref	Expression localization
MLH1	Ventana	M1	Pre-diluted	Mouse Monoclonal	790-4535	Nuclear
PMS2	Ventana	EPR3947	Pre-diluted	Rabbit monoclonal	790-4531	Nuclear
MSH2	Ventana	G219-1129	Pre-diluted	Mouse Monoclonal	790-4265	Nuclear
MSH6	Ventana	44	Pre-diluted	Mouse Monoclonal	790-4455	Nuclear

(51%) patients were men and 23 (49%) were women. The numbers according to tumor location, which were classified as right sided colon (from ileocecal colon to right colon angle), transversal colon, left sided colon (from descending colon to sigmoid colon), and rectum (from recto-sigmoid colon to distal rectum) were 19.15%, 4.25%, 14.89% and 61.71%, respectively. The numbers of well, moderately and poorly differentiation were 33, 13 and 1, respectively. Forty five of cases were nonmucinous, and remaining were mucinous. The TNM stage grouping was as follows: I (T1-4, N0, and M0) was 55.32% and II (T1-4, N^+, M^+) was 44.68%

Tableau 3 : variation des sensibilités et spécificités dans différentes séries de la littératureClinico-pathological data of the 47 CRC patients n, %

Clinico-pathologicalcharacteristics	n	%
Sex		
Female	23	49
Male	24	51
(Age (years		
≤ 60	19	40,42
> 60	28	59,58
Tumor location		
Right colon(RC)	9	19,15
Transversal colon (TC)	2	4,25
Left colon (LC)	7	14,9
Rectum (R)	29	61,71
Tumor grade		
Welldifferentiated (WD)	33	70,21
Moderatelydifferentiated (MD)	13	27,66
Poorlydifferentiated (PD)	1	2,13
Histological type		
none mucinousadenocarcinoma (NMA)	45	95,75
mucinousadenocarcinoma (MA)	2	4,25
Embols/peri-neuralinvasion		
Presence	24	51,06
Lack	20	42,55
Undetermined	3	6,39
TNM huddled		
I	26	55,32
II	21	44,68

MMR protein expression.

The frequency of abnormal expression of hMLH1, hMSH2, hMLH6 and PMS2 was 19.15%, 21.28%, 12.77%, and 19.15%, respectively (Fig.1 and Fig.2). Two same cases have been shown both abnormal for hMLH1 and PMS2 and five cases were abnormal for both hMSH2 and hMSH6. Twenty three of the 47 cases (70.21**%**) analyzed CRCs exhibited normal MMR protein expression. The relationship between clinical-pathological data and expression of MMR proteins of the tumors is summarized in Table 4. In our study, we found a significant association between the expression pattern of hMLH1 and age (*P*=0.02). Expression pattern of hMSH2 were significantly associated with age and TNM stage in respectively *P*=0.03 and *P*=0.02. Moreover, we found also a significant correlation between the immunohistochemical expression of hMSH6 and tumor grade (*P*=0.01).

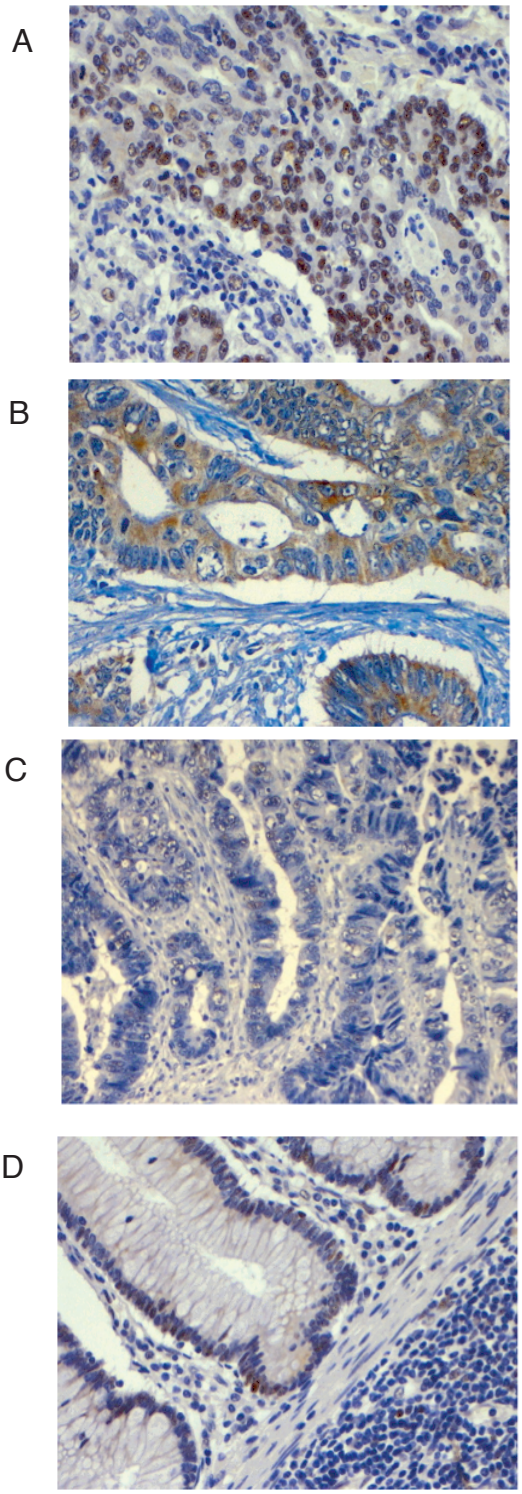

Figure 1: MSH2 and MSH6 IHC staining: a and d) Nuclear staining of lymphocytes in the stroma and normal mucosa, respectively, served as internal positive control (original magnification ×400), b) Adenocarcinoma with complete loss of MSH2 expression (original magnification ×400), a, c) Loss of MSH6 expression in tumor (original magnification ×200).

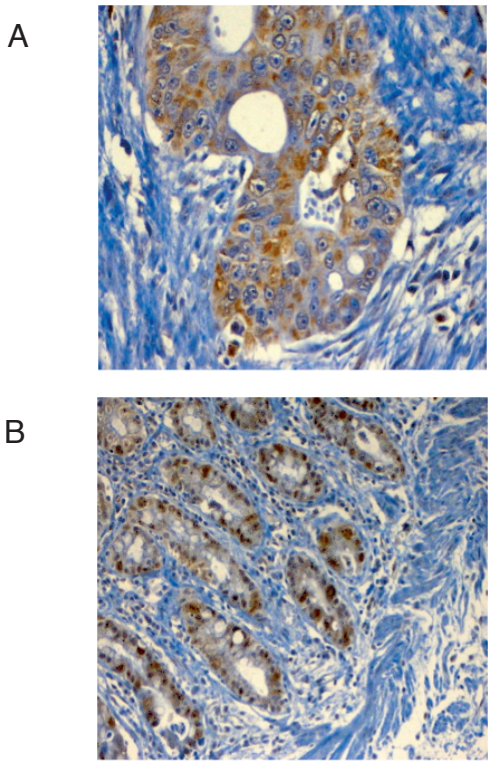

Figure 2 : IHC profile of MMR protein: (a) Positive nuclear staining of lymphocytes in stroma but loss of PMS2 expression in tumor epithelial cells (original magnification ×400). MLH1 shows a nuclear positivity in normal colorectal mucosa and in tumor epithelial cells (original magnification ×200).

Pattern and frequency of MSI.

Among our series, seven cases present positive Bethesda criteria. Two of them were MSI-H tumors. All the MSI tumors showed instability in two to five analyzed markers data. For all cases, the analysis of Microsatellite Instability was studied by DNA fragment analysis and has been confronted with the IHC results. We found that both methods of investigation were completely coherent. The relationship between MSI status and clinical and pathological features is summarized in Table 5.

DISCUSSION

Colorectal cancer is the most common type of cancer in worldwide. Its incidence is rapidly increasing [1, 2, 8] especially in developing countries. Microsatellite Instability analysis can be applied for diagnosis of HNPCC syndrome and for detection of MSI-H tumors in sporadic CRC. Besides, other tumor-specific markers such as*KRAS* and *BRAF* mutations are useful for both prognosis and directing adjuvant chemotherapy [9]. Moreover, MSI phenotype might be a useful molecular predictive marker for response to 5-FU adjuvant therapy [10-12] and genetic assessment of Lynch syndrome. International consensus meeting held in 1997 proposed a panel of two mononucleotides (Bat-25, Bat-26) and three dinucleotide markers (D2S123, D5S346 and D17S250) for use in uniform analysis of MSI [5, 13]. In molecular investigation, MSI-H tumors are showing instability at two or more of these markers, MSI-L tumors are showing at one or more repeat and MSS tumors are showing instability at none one of these repeats. Zhou et *al.* reported that Bat-26 identified in MSI status for 539 of 542 (99.5%) tumors analyzed [14]. According to our data, sensitivity for this mononucleotide repeats was 99%, but Bat-26 alone or with Bat-25 is insufficient for MSI-H screening. However, another way to detect MSI phenotypes is IHC staining. Herein, we have usedthis methodforanalysis of MMR proteins in primary tumors, invasive margin and normal mucosa. Fourteen cases showed loss of protein expressionof hMLH1, hMSH2, hMSH6 or PMS2(Table 4).Furthermore, our results are reinforced by others published data, support the evidence that loss of MMR protein expression plays an important role in the development of some colorectal carcinomas [15, 16]. It has been well established that MMR system plays a primary role during oncogenesis and tumor progression of approximately 15 to 25% of sporadic CRC and the majority of HNPCC cases [17]. Moreover, approximately in all published articles using IHC analysis, the MSI phenotype is assigned following the loss of expression of hMLH1 and/or hMSH2 in tumor [15]. However, 70.2% of cases were phenotypically stable (MSS) and the rest cases were instable (MSI). These results show a high frequency of instability in our set compared to those reported in some literature data. This frequency variation may be due to diverse factors like socio-demographic (late diagnosis, advanced age of person, etc.), psychosocial (lack of concern about the disease, etc.) and access to care. Furthermore, we studied the relationship between MMR protein expression profile and clinico-pathological characteristics of patients and we found a significance correlation between expression of hMLH1 and age (P=0.02) while hMSH2 expression pattern were significantly associated with age and TNM stage in respectively (P=0.03) and (P=0.02). Moreover, we found also a high significant correlation between the immunohistochemical expression of MSH6 with tumor grade (P=0.01) [5]. However, according to the review of literature, MSI tumors have a proximal location, poorly differentiated, mucinous carcinoma and intense peri- and intra-tumoral lymphocytic infiltration [10, 16, 18, 19]. Thus, when we evaluated the association between MSI phenotype of patients and their characteristics, we showed a high significant association only with embols/peri-neural invasion (P=0.01). Altered MisMatch Repair genes may be involved in mucin synthesis or degradation resulting in increased amount of mucin in these tumors compared to stable tumors. In our study, the results of molecular analysis of MSI status confirm those of immunohistochemistry with high sensitivity. In this

Table 4 : Histopathological associations with MMR expression

Histopathology (n=47)	MLH1				p value	PMS2				p value	MSH2				p value	MSH6				p value
	Positif		Negatif			Positif		Negatif			Positif		Negatif			Positif		Negatif		
	n	%	n	%		n	%	n	%		n	%	n	%		n	%	n	%	
Sex																				
F	20	86,96	3	13,04	0,29	19	82,61	4	17,39	0,76	20	86,96	3	13,04	0,28	21	91,3	2	18,7	0,66
M	18	75	6	25		19	79,17	5	20,83		17	70,83	7	29,17		20	83,33	4	16,67	
Age (years)																				
≤ 60	12	31,58	7	77,78	0,02	13	33,33	6	66,67	0,12	18	48,65	1	10	0,03	17	41,46	2	33,33	1
> 60	26	68,42	2	22,22		26	66,67	3	33,33		19	51,35	9	90		24	58,54	4	66,67	
Tumor location																				
RC	7	18,42	2	22,22	0,87	8	21,05	1	11,11	0,73	8	21,62	1	10	0,07	7	17,07	2	33,33	0,32
TC	2	5,26	0	0		2	5,26	0	0		2	5,4	0	0		2	4,88	0	0	
LC	6	15,79	1	11,11		5	13,16	2	22,22		3	8,11	4	40		5	12,2	2	33,33	
RS	23	60,53	6	66,67		23	60,53	6	66,67		25	67,57	5	50		27	65,85	2	33,33	
Tumor grade																				
WD	28	73,68	5	55,55	0,08	27	71,06	6	66,67	0,82	27	72,97	6	60	0,56	28	68,29	5	83,33	0,01
MD	11	28,95	2	22,22		10	26,32	3	33,33		9	24,32	4	40		13	31,71	0	0	
PD	0	0	1	1,23		1	11,12	0	0		1	2,71	0	0		0	0	1	16,67	
Histological type																				
NMA	37	97,37	8	88,89	0,34	36	94,74	9	100	1	36	97,3	9	90	0,38	40	97,56	5	83,33	0,24
MA	1	2,63	1	11,11		2	5,26	0	0		1	2,7	1	10		1	2,44	1	16,67	
EV/EP																				
Presence	22	57,89	2	22,22	0,38	20	52,63	4	44,44	0,7	21	56,76	3	30	0,26	22	53,66	2	33,33	0,64
Lack	16	42,11	4	44,44		15	39,47	5	55,56		14	37,84	6	60		17	41,46	3	50	
TNM huddled																				
I	22	57.89	4	44,44	0,48	23	60.53	3	33.33	0,26	24	64.86	2	20	0,02	24	58.54	2	33.33	0,38
II	16	42.11	5	55,56		15	39.47	6	66.67		13	35.14	8	80		17	41.46	4	66.67	

concept, the incidence rates of MSI phenotypes had been noted to differ from country to country or city to city. Microsatellite instabilityhad been reported to occur in 7 to 20 % of colon tumors. Of this fact, we reported an elevated rate of instability in our CRC series (MSI 29.8%).According our results, only the MSI-H phenotype is clinically significant and has better free-recurrence and overall survival compared to MSI-L/MSS phenotypes which they have the same clinical and prognostic valuebut no clinical significance. Besides, in sporadic stage II tumors, MSI-H has a resistance to alkylating agents and cisplatin[20]but they have better global survival and survival without second recurrence than stage III and IV tumors [21]. On the other hand, in the family forms marked by HNPCC syndrome, MSI-H is caused mainly by germinal mutations in *MLH1* or *MSH2* gene [22]. In this frame, individuals suspected with HNPCC syndrome now undergo routine colonoscopy, but many of them do not take regular screening and are therefore targeted to the development of cancer. The identification of patients with HNPCC is not only important for diagnosis but is also for prognosis, treatment, and prevention. On this occasion, it is necessary to start these new diagnostic strategies, based on collaboration between pathologists and molecular biologists by analyzing microsatellite status for each suspected person as a precautionary measure.Several methods allow identifying CRC with MSI as the molecular biology that is considered as the “gold standard” for the identification of this phenotype. It is based on the detection of instability in biomarkers within the DNA extracted from the neoplastic mucosa compared to that extracted from the normal mucosa [23-25]. For the diagnosis, a consensus conference organized by the National Institute of Health (NIH, Bethesda, USA) proposed the use of a minimum of five satellite markers chosen for their sensitivity and specificity. The second method is IHC which can demonstrate the loss of expression of MMR protein and target the causal gene of pathology [3, 22, 26]. With these techniques, three phenotypes can be identified: (i) Higher Microsatellite Instability when at least two or more of the studied markers are abnormal (MSI-H), (ii) lower microsatellite instability (MSI-L) when a single marker is considered as abnormal, and (iii) Microsatellite Stability (MSS). Only the MSI-H phenotype is clinically significant and has a good prognosis. In spite, the powerful of these methods, each one has its advantages and disadvantages. Immunohistochemistry has being applicable to any type of tissue, including frozen or fixed samples but in contrast, cannot, at present, detect the mutations that engender proteins whose function is distorted but structure is normal. Also, IHC can therefore be considered as a rapid, easier, and less costly alternative method for the detection of the mutator phenotype of CRCs, and it could be performed by laboratories as a routine diagnostic test to detect patients with MSI [27, 28]. The gold standard is PCR that has the advantage to obtain very reproducible results, but it cannot be used on some fixed tissue especially those fixed with Biotin liquid that block PCR reaction. At present, the various methods used in molecular biology for the identification and exploration of MSI phenotype in colorectal carcinoma are complementary: DNA fragment analysis for at least five markers, IHC using antibody against MMR protein in question and afterward target the corresponding gene and finely qPCR or NGS. We can note that nature of the samples can play an important role of detection of MSI phenotype especially for the extraction of DNA but immunohistochemistry can offer a diagnosis when the PCR failed due to inadequate fixation.

In summary, through this present study, the immunohistochemical analysis of MisMatch Repair protein expression is a practical and easy method for the routine detection of the MSI phenotype in patients with colorectal carcinoma.

References

1. Kin C KE, Poultsides GA, Visser BC, Jeffrey SS. Colorectal cancer diagnostics: biomarkers, cell-free DNA, circulating tumor cells and defining heterogeneous populations by single-cell analysis. Expert Rev Mol Diagn 2013;6:581–99.
2. Siegel R ND, Jemal A. Cancer statistics. CA Cancer J Clin 2013;63(1):11–30.
3. Dietmaier W WS, Bocker T, Kullmann F, Fishel R, Rüschoff J. Diagnostic microsatellite instability: definition and correlation with mismatch repair protein expression. Cancer Res 1997;57:4749–56.
4. JMD W. Epigenetic, mismatch repair genes and colorectal cancer. Ann R Coll Surg Engl 2005;87:15–20.
5. Sang-Bong Jung H-IL, Hoon-Kyu Oh, Im-Hee Shin and Chang-ho Jeon. Clinico-pathologic parameters for prediction of microsatellite instability in colorectal cancer. Cancer Res treat 2012;44(3):179-86.
6. Boland GM CG, Haynes AB, Chiang YJ, Chagpar R, Xing Y. Association between adherence to National Comprehensive Cancer Network treatment guidelines and improved survival in patients with colon cancer. Cancer Res. 2013;119(8):1593–601.
7. M K. Pathogenesis of microsatellite-unstable colorectal cancer. Evaluation of new diagnostic and therapeutic options. Pathologie. 2013;34:277–81.
8. Ferlay J SH, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010;127(12):2893–917.
9. Mostert B JY, Sieuwerts AM, Wang H, Bolt-de Vries J, Biermann K, et al.. KRAS and BRAF mutation status in circulating colorectal tumor cells and their correlation with primary and metastatic tumor tissue. Int J Cancer 2013;133(1):130–41.
10. Y N. Biologic behavior of microsatellite-unstable colorectal cancer and treatment with 5-fluorouracil. Isr Med Assoc J 2005;7(8):520–4.
11. Hemminki A MJ, Järvinen H, Aaltonen LA, Joensuu H. Microsatellite

instability is a favorable prognostic indicator in patients with colorectal cancer receiving chemotherapy. Gastroenterology 2000;119(4):921–8.
12. Elsaleh H IB. Microsatellite instability is a predictive marker for survival benefit from adjuvant chemotherapy in a population based series of stage III colorectal carcinoma. Clin Colorectal Cancer 2001;1(2):104–9.
13. Rodriguez-Bigas MA BC, Hamilton SR, Henson DE, Jass JR, Khan PM, et al. . A national cancer institute workshop on hereditary nonpolyposis colorectal cancer syndrome: meeting highlights and Bethesda guidelines. J Natl Cancer Inst 1997;89:1758-62.
14. Zhou XP HJ, Li YJ, Senuca R, Cameiro F, Sobrinhi-Simoes M, et al.. Determination of the replication error phenotype in human tumors without the requirement for matching normal DNA by analysis of mononucleotide repeat microsatellites. Gene chromosomes cancer 1998; 21(2):101-7.
15. Mensenkamp AR VI, van Zelst-Stams WA, Goossens M, Ouchene H, Hendriks-Cornelissen SJ, et al. . Somatic mutations in MLH1 and MSH2 are a frequent cause of mismatch-repair deficiency in Lynch syndrome-like tumors. Gastroenterology. 2013;146(3):643-6.
16. Aissi S BM, Zerimech F, Kourda N, Moussa A, Manai M, et al. . Somatic molecular changes and histo-pathological features of colorectal cancer in Tunisia. World J Gastroenterol 2013;19(32):5286–94.
17. Marra G JJ. DNA mismatch repair and colon cancer. Adv Exp Med Biol 2005;570:85–123.
18. Edmonston TB CK, Burkholder S, Barusevicius A, Rose D, Kovatich AJ, et al. Colorectal carcinomas with high microsatellite instability: defining a distinct immunologic and molecular entity with respect to prognostic markers. Hum Pathol 2000;31(12):1506–14.
19. Rigau V NSB, Sylviane O, Francois P, NajatM, Yann P, et al. . Microsatellite instability in colorectal carcinoma: the comparison of immunohistochemistry and molecular biology suggests a role for hMLH6 immunostaining. Arch Pathol Lab Med 2003;127:694–700.
20. Roth AD D, Tejpar S, Yan P, Klingbiel D, Fiocca R, et al. . Integrated analysis of molecular and clinical prognostic factors in stage II/III colon cancer. J Natl Cancer Inst 2012;104(21):1635–46.
21. Kang BW KJ, Lee SJ, Chae YS, Moon JH, Sohn SK, et al.. Clinical significance of microsatellite instability for stage II or III colorectal cancer following adjuvant therapy with doxifluridine. Med Oncol 2011;28(1):214-8.
22. Malhotra P AM, Kochhar R, Ahmad S, Vaiphei K, Mahmood S. Promoter methylation and immunohistochemical expression of hMLH1 and hMSH2 in sporadic colorectal cancer: a study from India. Tumor Biol 2014;35(4):3679-87.
23. Suraweera N DA, Reperant M, Vaury C, Furlan D, Leroy K, et al. . Evaluation of tumor microsatellite instability using five quasimonomorphic mononucleotide repeats and pentaplex PCR. Gastroenterology 2002;123:1804–11.
24. Lech G SR, Krasnodebski IW The role of tumor markers and biomarkers in colorectal cancer. Neoplasma 2014;61(1):1-8.
25. Patil DT BM, Portier BP, Fraser CR, Plesec TP, Liu X. A five-marker panel in a multiplex PCR accurately detects microsatellite instability-high colorectal tumors without control DNA. Diagn Mol Pathol 2012;21(3):127–33.
26. Lawes DA PT, SenGupta S, Boulos PB. The role of MLH1, MSH2 and MSH6 in the development of multiple colorectal cancers. Br J Cancer 2005;93:472–7.
27. Lanza G GR, Maestri I, Santini A, Matteuzzi M, Cavazzini L. Immunohistochemical pattern of MLH1/MSH2 expression is related to clinical and pathological features in colorectal adenocarcinomas with microsatellite instability. Mod Pathol 2002;15(7):741-9.
28. Jung KW PS, Won YJ, Kong HJ, Lee JY, Park EC, et al. . Prediction of cancer incidence and mortality in Korea. Cancer Res Treat 2011;43:12-8.