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Bat-26 is Associated with Clinical Stage and Lymph Node Status in Schistosomiasis Associated Bladder Cancer

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Abstract **Background:** Carcinoma of the urinary bladder is the most common malignancy in Egypt in which infection with Schistosoma haematobium is prevalent. This type of malignancy has different characteristics than that observed in the western world. We studied 5 microsatellite instability markers in exfoliated urinary samples from schistosomiasis associated bladder cancer cases and compared the results to the clinicopathological data of the patients. We also studied the sensitivity and specificity of these markers. **Material and methods:** The study included 32 histopathologically diagnosed BC patients and 11 age and sex matched normal controls. DNA was extracted from the exfoliated urinary cells and PCR was done and the product was separated by 12% PAGE. BAT-25, BAT-26, NR-21, NR-22 and NR-24 mutations were evaluated. **Results:** MSI in at least one marker was observed in 71.87% of BC cases. Mutations in BAT 25 (21.4%), BAT-26 (12.9%), NR-21 (58%), NR-22 (35.5%) were observed. No mutation was observed for NR-24 (0%). BAT 26 mutation was significantly associated with both tumor stage (P 0.03) and L.N metastasis (p 0.005). BAT25 was 21.4% sensitive and 100% specific. BAT26 mutation was 12.9% sensitive and 100% specific. NR21 was 58% sensitive and 100% specific. NR22 was 35.5% sensitive and 91% specific. NR24 was 0% sensitive and 100% specific for diagnosis. **Conclusions:** BAT26 mutation is significantly associated with clinical stage and lymph node metastasis in bladder cancer. All the tested markers showed high specificity. NR21 showed the highest sensitivity. More studies are needed to detect the prognostic significance of MSI in bladder cancer.

Keywords Bladder Cancer, Schistosomiasis, MSI, Clinicopathological data

1. Introduction

Bladder cancer is the seventh most common malignancy in men worldwide, with 297,300 new cases estimated in 2008 [1]. Rates of bladder cancer are highest in Europe, North America, and Northern Africa, where Egyptian men have the highest incidence (37.1 per 100,000 person-years) and mortality rates (16.3 per 100,000 person-years) [2]. Transitional cell carcinoma (TCC) is the most common histopathological type, occurring in approximately 90% of all bladder cancers in Western countries, with a peak incidence in the seventh decade of life [3-5].

In Egypt, urinary bladder cancer incidence is among the highest worldwide [6, 7]. Chronic bladder infection with *Schistosoma haematobium*, the trematode causing urinary schistosomiasis as well as smoking are the most important risk factors for bladder cancer in Egypt [8, 9].

Currently Squamous cell carcinoma represents about 30% of all bladder cancer cases [9, 10] where there is a significant shift from 60-70% of bladder cancer cases reported before the 1980's. This may be due to *Schistosoma hematobium* eradication campaigns [8, 11].

Radical cystectomy is still the treatment of choice for most of the cases [12]. However, the 5 year survival rate is still not satisfactory (13). Because most of the patients are presented with advanced stage of the disease, the introduction of adjuvant or neoadjuvant chemotherapy does not add too much to the current survival figures [14]. Extensive studies are clearly needed to discover the molecular abnormalities that may lead to bladder cancer formation and resistance to therapy.

Genomic instability is a characteristic feature of almost all major human tumors. Numerous genetic and molecular alterations occur in TCC of the bladder [15]. Genetic instability by itself can lead to the development of bladder cancer [16].

Microsatellites are short repeats of nucleotide sequences

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of DNA, consisting of 1–5 nucleotides [17]. Because of their repeat structure, microsatellites are prone to replication errors. These errors are repaired by the DNA mismatch repair pathway (MMR) [18]. However, this pathway may fail resulting in microsatellite instability ((MSI).

Tumor is classified as MSI-high (MSI-H) if it shows instability in at least 2 of 5 markers; MSI-low (MSI-L) if 1 of 5 and microsatellite stable (MSS) if none of 5. [19]

Several studies have reported the presence of MSI in urothelial cell carcinoma (UCC) of the bladder [20] [21]. MSI has also been found in many cancers including gall bladder cancer, ovarian cancer and colorectal cancer [22-24].

The assessment of MSI (BAT25, BAT26, NR21, NR22 as well as NR24) status can help in establishing a clinical prognosis and may predict tumor response to chemotherapy [25]. MSI can also be used as a prognostic marker for the detection of many human cancers [26].

The aim of the present study is to detect the occurrence of the 5 microsatellite instability markers BAT-25, BAT-26, NR-21, NR-22 and NR-24 in exfoliated urinary samples from schistosomiasis associated bladder cancer cases and comparing the results to the clinicopathological data of the patients and testing the sensitivity and specificity of these markers for the detection of the disease.

2. Patients and Methods

2.1. Patients

The present study was conducted according to the guidelines of the ethical principles outlined in the declaration of Helsinki. The study was approved by the ethical committee of the National Cancer Institute, Cairo University as well as Theodor Bilharz Research Institute, Egypt. The study included a total of 32 bladder cancer cases attending to both institutions and 11 age and sex matched normal controls.

2.2. Inclusion Criteria

Only new cases with clinically diagnosed bladder cancer were involved in the study. The patients did not receive any type of therapy. Selection of the patients was carried out depending on the results of both endoscopy and the histopathological examination of the removed samples.

After approval of the ethical committee, urine samples were collected from the patients before being subjected to any type of therapy. For those who had radical cysctectomy as a primary treatment modality, diagnosis of bladder cancer was confirmed by histo-pathological examination of the removed tumor tissues by 2 independent pathologists.

2.3. Methods

Voided Morning urine samples were collected from patients and normal controls into 50 ml sterile Falcon tubes before therapy and transferred to the laboratory. Samples were centrifuged at 1200 rpm for 20 minutes. The supernatant was decanted and the pellet was re-suspended in 1x pbs (PH7.2) and centrifuged again. The supernatant was removed and the pellet was stored at -80 until the DNA extraction.

2.4. DNA Extraction and Polymerase Chain Reaction

Total DNA was extracted from the cells using the Abott kit. (Abbott Park, Illinois, U.S.A), according to the manufacturer's protocol. The DNA samples were coded and stored at – 20°C until analysis. Polymerase chain reaction was performed in MJ Research PTC-100 machine. The following set of primer sequences for BAT-25, BAT-26, NR-21, NR-22 and NR-24 were used (27) as shown in Table 1.

A total of 45 cycles of PCR amplification were used, the cycles were as follows denaturation for 95°C for 5 minutes, 95°C for 30 sec annealing for 30 sec at 55°C and extension for10 minute at 72°C, Followed by one step of final extension for 10 min. at 72°C.

The amplified PCR products were electrophoresed on 12% PAGE polyacrylamide gel electrophoresis (3 h 150 V) and visualized by ethidium bromide staining. The gel image was analyzed using Cleaver micro DOC gel documentation system.

The presence of extra or shifted bands in tumor DNA compared to any normal DNA was classified as MSI according to Catto et al., 2003; Turyn et al., 2006. [28] [29]

Interpretation of results is based on the classification of allelic size variation in ≤2 of 5 markers as MSS and in ≥3 of 5 markers as MSI-H according to Suraweera et al., 2002 [27], variations of ≥3 bp for BAT-25, NR21, NR-22, and NR-24 alleles and of ≥4 bp for BAT-26 were considered to represent polymorphisms or somatic alterations.

Table 1. Primer sequences for the 5 mononucleotide markers used for MSI detection

Marker	Sense primer (5′→3′)	Antisense primer (5′→3′)	PCR product size
BAT25	5- tcgcctccaagaatgtaagt-3	5- tctgcattttaactatggctc-3	120
BAT26	5- tgactacttttgacttcagcc-3	5-aaccattcaacatttttaaccc-3	124
NR21	5- taaatgtatgtctcccctgg-3	5- attcctactccgcattcaca-3	103
NR22	5-gaggcttgtcaaggacataa-3	5-aattcggatgccatccagtt-3	142
NR24	5-ccattgctgaattttacctc-3	5- attgtgccattgcattccaa-3	132

2.5. Statistical Analysis

Statistical Package for the Social Sciences for Windows (version 12.0, SPSS, Chicago, IL, USA) was used for data management. Chi‑square and Fisher's exact test were used for testing proportion independence. Data were presented as mean ± standard deviation (or median) and frequencies (%). P value is significant at 0.05 levels.

Table 2. Clinicopathological characteristics of the bladder cancer patients involved in the study

Patient Characteristics		Number	Percent
Mean Age	59.9	32	100%
Sex	Male	23	71.9 %
	Female	9	28.1 %
Histological Grade	I	1	5 %
	II	11	55 %
	III	8	40%
	NA	12	
Histo-pathological type	TCC	19	90.4 %
	SCC	1	4.8 %
	SCC+TCC	1	4.8 %
	NA	11	
Clinical stage	P1	11	61%
	P2	5	28%
	P3	2	11%
	NA	14	
Bilharziasis	+ve	6	27 %
	-ve	16	73 %
	NA	10	
Lymph node involvement	Negative	7	87.5 %
	Positive	1	12.5 %
	NA	24	

3. Results

Thirty two cases were involved in the study. The histopathological data of only 22 cases were evaluable the remaining 10 cases were histpathologically inavailable. Nine patients were females and 23 were males. The mean age was 59.1 years (± 10.7). The histopathological features of the patients are shown in (table 2).

Out of 32 cases, instabilities in at least one marker were detected in 23 cases (71.8%).

BAT25 mutation was detected in 3 out of 14 tested samples (21.4%). BAT26 mutation was detected in 4 out of 31 tested samples (12.9%). NR21 mutation was detected in18 out of 31 samples (58%). Mutation in NR22 was detected in 11 out of 31 samples (35.5%). However, we did not find any mutations in NR24 for the 32 tested samples (0%). (Table 3)

Table 3. Microsatellite instability status of the 32 studied bladder cancer cases

Microsatellite Markers	Normal samples	Mutant samples	Total number
BAT25	11	3 (21.4%)	14
BAT 26	27	4 (12.9%)	31
NR21	13	18(58%)	31
NR22	20	11(35.5%)	31
NR24	32	0 (0%)	32

By studying the correlation between the 5 markers and the histopathological data of the patients, we did not find any significant correlation between the histopathological data and any of the markers including BAT25, NR21, NR22, and NR24. However, BAT26 was significantly associated to clinical stage (P 0.031) and lymph node metastasis (P 0.005). (Table 4)

Table 4. Correlation between microsatellite instability of the BAT26 and the different clinico-pathological data of the patients

Characteristic	BAT26		TOTAL	PVALUE
	N	M		
M	15	1	21	0.429
F	4	1		
GI	1	0	19	0.079
GII	11	0		
GIII	5	2		
TCC	16	2	20	0.648
SCC	1	0		
TCC+SCC	1	0		
PI	11	0	17	0,031*
PII	3	1		
PIII	1	1		
Bilh +ve	5	0	21	0.571
Bilh -ve	14	2		
L.N +ve	0	1	8	0.005*
L.N -ve	7	0		

When we studied the sensitivity and specificity of the 5 markers for diagnosis of bladder cancer, we found that BAT25 is 21.4% sensitive and 100% specific. BAT26 is

12.9% sensitive and 100% specific. NR21 is 58% sensitive and 100% specific. NR22 is 35.5% sensitive and 90.91% specific. However NR24 is 0% sensitive and 100%specific for diagnosis of bladder cancer. (Table 5)

Table 5. The sensitivity and specificity of the 5 markers for diagnosis of bladder cancer

Marker	Bladder Cancer	Normal	Sensitivity	Specificity
BAT25				
Mutant	3	0		
Normal	11	11	21.4%	100%
Total	14	11		
BAT26				
Mutant	4	0		
Normal	27	11	12.9%	100%
Total	31	11		
NR21				
Mutant	18	0		
Normal	13	11	58 %	100%
Total	31	11		
NR22				
Mutant	11	1		
Normal	20	10	35.5%	90.91%
Total	31	11		
NR24				
Mutant	0	0	0%	
Normal	32	11		100%
Total	32	11		

4. Discussion

Bladder cancer in Egypt is still a major problem where patients presented with advanced stage of the disease. Also the survival rate is still low. Early detection of the disease is needed to detect the early stages of the disease. Many advances have been made to discover a reliable marker that may detect the disease in its early stages [30] [31]. Microsatellite instability have been studied in many cancers such as colorectal cancer, melanoma, endometrial carcinoma, breast cancer and bladder cancer [32-36]. In the present work we studied the MSI in exfoliated urinary cells for 34 schistosomiasis associated bladder cancer then correlated the results to the histopathological criteria of the patients.

The 5 mononucleotide markers have been studies in many types of cancer. Wong et al used BAT25, BAT26, NR21, NR22 and NR24 in endometrial carcinoma. They concluded that the best markers within the NCI panel are the mononucleotide repeats BAT-25 and BAT-26 [37]. Another study by Xicola et al was conducted on 1058 colorectal tumors, they compared the performance of two panels of markers, and they found that the pentaplex panel of mononucleotide repeats performs better than the NCI panel for the detection of mismatch repair–deficient tumors. Simultaneous assessment of the instability of BAT26 and NR24 is as effective as use of the pentaplex panel for diagnosing mismatch repair deficiency [38].

Zulueta et al. found that microsatellite instabilities are present as an apparently early event in the development of bladder cancer. [39]

Our data indicated that mutation was detected in 4 microsatellites these are BAT25, BAT26, NR21, and NR22. However the remaining marker NR24 was normal. Vaish et al studied MSI in 44 superficial bladder cancer cases; he found that MSI play important role in evolution, initiation and progression in bladder tumors. [40]

Although MSI have extensively studied in many types of cancer, there was a great variability between the results from study to another.

Artus et al found that MSI has been observed in only one of the 17 bladder cancer studied cases [41]. However Bonnal et al investigated the prevalence of the microsatellite instability using a panel of six microsatellite markers where 3 mononucleotide repeats (BAT26, TGF betaRII, and BAX) were studied in 33 TCC samples and in four bladder cancer cell lines. They found no alteration was detected either in the 33 TCC samples analyzed or in the four bladder cancer cell lines [42].

This variability may results from the etiology of the diseases from one population to another.

In order to detect the clinical significance of MSI, we compared the results to the clinicopathological criteria of the patients. Among all the clinical data studied, only BAT26 was significantly associated to high clinical stage and lymph node metastasis.

Vaish et al. 2005 examined BAT26 on 44 bladder superficial tumors, the results were compared to the clinico-pathological data of the patients, BAT26 was significantly associated to clinical stage [40]. The same author on 2003 found that the instability with BAT-26 was found to be 20%. Eighty-three percent of the unstable urinary bladder cancers were found to have a high grade in a superficial group, whereas only 27% MSI +ve were muscle invasive cancers [43]. Also Burgos et al studied microsatellite instability on DNA extracted from exfoliated urinary samples from 165 bladder cancer samples. They found that the biggest number in MSI was found in superficial tumors and GI-GII tumors [44]. However, Catto et al. 2003 did not find any significance between MSI and tumor stage in TCC of 84 bladder cancer cases [45]. For other types of cancer, Beghelli S et al. found that gastric cancer is significantly associated to lower stage; he also found that only stage II cancers showed a significant effect of MSI status on survival [46]. However, Ju etal. found that there is no significant association between MSI status and clinical stage in endometrial adenocarcinoma [47]. The same result was found in esophageal cancer where there was no significance between BAT26 and any of the clinicopathological data including clinical stage [48].

Our Study indicated that there is a significant association between BAT26 instability and lymph node metastasis.

Kazama et al. found that microsatellite instability in poorly differentiated colorectal adenocarcinoma is significantly associated with lymph node involvement. [49].

Also Malesci et al. found a strong association between MSI and decreased lymph node metastasis in colorectal cancer [50]. In gastric cancer, BAT26 alteration was highly correlated with less lymph node metastasis [51].

From the above data it seems that MSI instability is different from tumor to another. Regarding the bladder cancer, the data from our study as well as the other studies indicate that MSI especially BAT26 is almost present in both superficial and invasive bladder cancer. Regarding the correlation between MSI and the clinicopathological features of bladder cancer, although our study showed a significant association between BAT26 and clinical stage and lymph node metastasis, but we cannot reach a final conclusion regarding the association of MSI to the other clinicopathological criteria which may be due to the low number of cases in our study.

5. Conclusions

BAT26 mutation is significantly associated with clinical stage and lymph node metastasis in bladder cancer. All the tested markers showed high specificity for the detection of the disease. Among the 5 markers, NR21 showed the highest sensitivity. More studies are needed to detect the prognostic significance of MSI in schistosomiasis associated bladder cancer.

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Competing Interests

We have read and understood the journal policy on disclosing conflicts of interest and we declare that we have no financial competing interests.

REFERENCES

[1] Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM.GLOBOCAN 2008 v1.2, Cancer Incidence and Mortality Worldwide: IARC Cancer Base No. 10 [Internet] Lyon, France: International Agency for Research on Cancer; 2010. Available at:http://globocan.iarc.fr.

[2] Murta-Nascimento C, Silverman DT, Kogevinas M, García-Closas M, Rothman N, Tardón A, García-Closas R, Serra C, Carrato A, Villanueva C, Dosemeci M, Real FX, Malats N. Risk of bladder cancer associated with family history of cancer: do low-penetrance polymorphisms account for the increase in risk?. Cancer Epidemiol Biomarkers Prev. 2007 Aug; 16(8):1595-600.

[3] Mostafa MH, Sheweita SA, O'Connor. Relationship between schistosomiasis and bladder cancer. PJ Clin Microbiol Rev. 1999 Jan; 12(1):97-111.

[4] Burnham N. Bladder cancer: detection, prevention, and therapeutics. Am Pharm. 1989 Jun; NS29(6):33-8.

[5] La Vecchia C, Negri E, D'Avanzo B, Savoldelli R, Franceschi S. Genital and urinary tract diseases and bladder cancer. Cancer Res. 1991 Jan 15; 51(2):629-31..

[6] Martine Ploeg, Katja K. H. Aben, and Lambertus A. Kiemeney. The present and future burden of urinary bladder cancer in the world. World J Urol. 2009 Jun; 27(3): 289–293.

[7] GLOBOCAN [Accessed 3-1-2012]; Cancer facts in Egypt. 2008. http://globocan.iarc.fr/factsheet.asp.

[8] Felix AS, Soliman AS, Khaled H, Zaghloul MS, Banerjee M, El-Baradie M, El-Kalawy M, Abd-Elsayed AA, Ismail K, Hablas A, Seifeldin IA, Ramadan M, Wilson ML. The changing patterns of bladder cancer in Egypt over the past 26 years. Cancer Causes Control. 2008 May; 19(4):421-9.

[9] Zheng YL, Amr S, Saleh DA, Dash C, Ezzat S, Mikhail NN, Gouda I, Loay I, Hifnawy T, Abdel-Hamid M, Khaled H, Wolpert B, Abdel-Aziz MA, Loffredo CA. Urinary bladder cancer risk factors in Egypt: a multicenter case-control study. Cancer Epidemiol Biomarkers Prev. 2012 Mar; 21(3):537-46.

[10] Wolpert BJ, Amr S, Saleh DA, Ezzat S, Gouda I, Loay I, Hifnawy T, Abdel-Hamid M, Mikhail NN, Zhan M, Zheng YL, Squibb K, Abdel-Aziz MA, Zaghloul MS, Khaled H, Loffredo CA. Associations differ by sex for catechol-O-methyltransferase genotypes and bladder cancer risk in South Egypt. Urol Oncol. 2012 Nov-Dec; 30(6):841-7.

[11] Rollinson D1, Knopp S, Levitz S, Stothard JR, Tchuem Tchuenté LA, Garba A, Mohammed KA, Schur N, Person B, Colley DG, Utzinger J. Time to set the agenda for schistosomiasis elimination. Acta Trop. 2013 Nov;128(2):423-40.

[12] Ghoneim MA, Abdel-Latif M, el-Mekresh M, Abol-Enein H, Mosbah A, Ashamallah A, el-Baz MA. Radical cystectomy for carcinoma of the bladder: 2,720 consecutive cases 5 years later. J Urol. 2008 Jul;180(1):121-7.

[13] Zaghloul MS. Adjuvant and neoadjuvant radiotherapy for bladder cancer: revisited. Future Oncol. 2010 Jul; 6(7): 1177-91.

[14] Khaled HM, Shafik HE, Zabhloul MS, Ghoneim M, Saber RA, Manie M, Enein HA, Megeed HA,Mansur O, Sherbini ME, Mahran TZ, Kalawee ME, Badran A, Ramadan SM.. Gemcitabine and cisplatin as neoadjuvant chemotherapy for invasive transitional and squamous cell carcinoma of the bladder: effect on survival and bladder preservation. Clin Genitourin Cancer. 2014 Oct;12(5):e233-40.

[15] Catto JW, Meuth M, Hamdy FC. Genetic instability and transitional cell carcinoma of the bladder. BJU Int 2004; 93:19-24.

[16] Schabath MB, Spitz MR, Grossman HB, Zhang K, Dinney CP, Zheng PJ, et al. Genetic instability in bladder cancer assessed by the comet assay. J Natl Cancer Inst 2003;95:540-7.

[17] H. Ellegren, Microsatellites: simple sequences with complex evolution. Nat Rev Genet, 5 (2004), pp. 435–445.

[18] W.B. Coleman, G.J. Tsongalis: Molecular mechanisms of human carcinogenesis. EXS (2006), pp. 321–349

[19] Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN, Srivastava S. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res, 58 (1998), pp. 5248–5257.

[20] Mongiat-Artus P1, Miquel C, van der Aa M, Buhard O, Hamelin R, Bangma C, Teillac P, van der Kwast T,Praz F. Infrequent microsatellite instability in urothelial cell carcinoma of thebladder in young patients. Eur Urol. 2006 Apr;49(4):685-90.

[21] Seripa D, Parrella P, Gallucci M, Gravina C, Papa S, Fortunato P, et al. Sensitive detection of transitional cell carcinoma of the bladder by microsatellite analysis of cells exfoliated in urine. Int J Cancer 2001;95:364-9.

[22] Boland CR, Goel A. Microsatellite instability in colorectal cancer. Gastroenterology 2010;138:2073-87.e3.

[23] Buller RE, Shahin MS, Holmes RW, Hatterman M, Kirby PA, Sood AK. p53 mutations and microsatellite instability in ovarian cancer: Yin and yang. Am J Obstet Gynecol 2001;184:891-902.

[24] Yoshida T, Sugai T, Habano W, Nakamura S, Uesugi N, Funato O, et al. Microsatellite instability in gallbladder carcinoma: Two independent genetic pathways of gallbladder carcinogenesis. J Gastroenterol 2000;35:768-74

[25] Jeffery W. Bacher, , Laura A. Flanagan, Regenia L. Smalley, Nadine A. Nassif, Lawrence J. Burgart, Richard B. Halberg, Wael M. Abdel Megid, and Stephen N. Thibodeau. Development of a Fluorescent Multiplex Assay for Detection of MSI-High Tumors. Dis Markers. 2004; 20(4-5): 237–250.

[26] Yamamoto Y, Matsuyama H, Kawauchi S, Furuya T, Liu XP, Ikemoto K, et al. Biological characteristics in bladder cancer depend on the type of genetic instability. Clin Cancer Res 2006;12:2752-8.

[27] Suraweera N1, Duval A, Reperant M, Vaury C, Furlan D, Leroy K, Seruca R, Iacopetta B, Hamelin R. Evaluation of tumor microsatellite instability using five quasimonomorphic mononucleotide repeats and pentaplex PCR. Gastroenterology. 2002 Dec;123(6):1804-11.

[28] James WF Catto, Abdel-Rahmene Azzouzi, Najla Amira, et al. Distinct patterns of microsatellite instability are seen in tumours of the urinary tract Oncogene (2003) 22, 8699–8706.

[29] Jacek Turyn, Marcin Matuszewski and Beata Schlichtholz. Genomic instability analysis of urine sediment versus tumor tissue in transitional cell carcinoma of the urinary bladder. Oncology Reports 15: 259-265, 2006.

[30] Khaled HM, Abdel-Salam I, Abdel-Gawad M, et al. Evaluation of the BTA tests for the detection of bilharzial related bladder cancer: the Cairo experience. Eur Urol. 2001 Jan;39(1):91-4.

[31] Abdel-Salam IM, Khaled HM, Gaballah HE, Mansour OM, Kassem HA, Metwaly AM. Telomerase activity in bilharzial bladder cancer. Prognostic implications. Urol Oncol. 2001 Jul;6(4):149-153.

[32] Chen WS, Chen JY, Liu JM, et al. Microsatellite instability in sporadic-colon-cancer patients with and without liver metastases. Int J Cancer. 1997 Aug 22;74(4):470-4.

[33] Hussein MR, Haemel AK, Albert DM, Wood GS. Microsatellite instability and alterations of mismatch repair protein expression in choroidal melanomas. Arch Ophthalmol. 2005 Dec;123(12):1705-11.

[34] Diab AM, Tawfeek TA, Moeity F, Elsammak M. . Mutations in exons 6 and 7 of TP53 gene correlate positively with serum tumor necrosis factor alpha independent of microsatellite instability in BAT26 gene in Egyptian patients with endometrial carcinoma. Int J Biol Markers. 2006 Jul-Sep; 21(3):184-9.

[35] Zekri AR, Bahnassi AA, Bove B, et al. Allelic instability as a predictor of survival in Egyptian breast cancer patients. Int J Oncol. 1999 Oct;15(4):757-67.

[36] Danaee H, Nelson HH, Karagas MR, et al. Microsatellite instability at tetranucleotide repeats in skin and bladder cancer. Oncogene. 2002 Jul 25;21(32):4894-9.

[37] Yick Fu Wong, Tak Hong Cheung, Keith Wing Kit Lo, et al. Detection of microsatellite instability in endometrial cancer: advantages of a panel of five mononucleotide repeats over the National Cancer Institute panel of markers. Carcinogenesis, vol.27 no.5 pp.951–955, 2006.

[38] Rosa M. Xicola, Xavier Llor, Elisenda Pons, et al. Performance of Different Microsatellite Marker Panels for Detection of Mismatch Repair–Deficient Colorectal Tumors. J Natl Cancer Inst Volume 99, Issue 3Pp. 244-252.

[39] Mirella Gonzalez-Zulueta, J. Michael Ruppert, Kaori Tokino, et al. Microsatellite instability in Bladder Cancer. Cancer Research 53, 5620-5623. December l. 1993.

[40] Minal Vaish13, Anil Mandhani2, RD Mittal2 and Balraj Mittal1. Microsatellite instability as prognostic marker in bladder tumors: a clinical significance. BMC Urology 2005 Jan 12;5:2.

[41] Pierre Mongiat-Artus, Catherine Miquel, Madelon van der Aa, et al. Infrequent Microsatellite Instability in Urothelial Cell Carcinoma of the Bladder in Young Patients. European Urology. Eur Urol. 2006 Apr;49(4):685-90.

[42] C Bonnal, V Ravery, M Toublanc, et al. Absence of microsatellite instability in transitional cell carcinoma of the bladder. Urology. 2000 Feb;55(2):287-91.

[43] Vaish M, Mishra SK, Mandhani A, Mittal RD, Mittal B. Assessment of microsatellite instability in bladder and thyroid malignancies. Teratog Carcinog Mutagen. 2003; Suppl 1: 255-65.

[44] Molina Burgos R, Millán Salvador JM, Oltra Soler JS, Jiménez Cruz JF. Microsatellite analysis in exfoliated cells from urinary sediment. Its utility for the detection of bladder cancer. Comparison with urinary cytology]. Actas Urol Esp. 2003 Sep;27(8):618-28.

[45] James W. F. CATTO, George Xinarlanos, Julian L. Burton, Mark Meuth and Freddie C. Hamdy. Differential expression of hMLH1 and hMSH2 is related to bladder cancer grade, stage and prognosis but not microsatellite instability. Int. J. Cancer 2003, 105, 484 – 490.

[46] Beghelli S, de Manzoni G, Barbi S, et al. Microsatellite

instability in gastric cancer is associated with better prognosis in only stage II cancers. Surgery. 2006 Mar;139(3):347-56.

[47] Ju W, Park HM, Lee SN, Sung SH, Kim SC. Loss of hMLH1 expression is associated with less aggressive clinicopathological features in sporadic endometrioid endometrial adenocarcinoma. J Obstet Gynaecol Res. 2006 Oct; 32(5):454-60.

[48] Kulke MH, Thakore KS, Thomas G, et al. Microsatellite instability and hMLH1/hMSH2 expression in Barrett esophagus-associated adenocarcinoma. Cancer 2001 Apr 15; 91(8):1451-7.

[49] Yoshihiro Kazama, Toshiaki Watanabe, Takamitsu Kanazawa, Junichiro Tanaka, Toshiaki Tanaka, and Hirokazu Nagawa. Microsatellite instability in poorly differentiated adenocarcinomas of the colon and rectum: relationship to clinicopathological features. J Clin Pathol. Jun 2007; 60(6): 701–704.

[50] Malesci A, Laghi L, Bianchi P, et al. Reduced likelihood of metastases in patients with microsatellite-unstable colorectal cancer. Clin Cancer Res. 2007 Jul 1;13(13):3831-9.

[51] Wu MS, Lee CW, Sheu JC, et al. Alterations of BAT-26 identify a subset of gastric cancer with distinct clinicopathologic features and better postoperative prognosis. Hepatogastroenterology . 2002 Jan-Feb;49(43):285-9.