

Role of Loss of Heterozygosity on Chromosomes 8 and 9 in the Development and Progression of Cancer Bladder

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ABSTRACT

Background: Loss of heterozygosity (LOH) in tumor samples is believed to be a marker for the absence of a functional tumor suppressor gene. Non-random chromosome deletion and LOH at specific chromosomal regions are identified in a number of common human cancers including carcinoma of the bladder, which is considered the most predominant cancer in Egypt due to the prevalence of schistosomiasis.

Purpose: The main objective of the present study is to clarify the role of chromosomes 8 and 9 in the establishment and/or progression of schistosomiasis-related bladder cancer through detection of LOH of 8 microsatellite markers on both chromosomes. It also aims to compare the LOH pattern of the tested markers between schistosomiasis-associated and non schistosomiasis-associated bladder cancer.

Material and Methods: To achieve this purpose, DNA was extracted from the tumor specimens and the corresponding peripheral blood samples of 42 primary bladder cancer patients (schistosomal and non schistosomal). Twenty nine of these were diagnosed as squamous cell type (SCC), 11 were transitional (TCC), and 2 were adenocarcinoma (with different stages and grades). LOH at chromosomes 8 and 9 was evaluated for 8 highly polymorphic microsatellite markers distributed at different regions of both chromosomes using the dinucleotide repeat-PCR technique.

Results: The overall percentage of LOH in chromosome 8 was 74% in at least one marker. The highest incidence of LOH was recorded for D8S84 (41%) followed by 37% for D8S87, 29% for D8S85, and 25% for D8S88. Deletions at chromosome 8 were shown to be associated with high grade of the tumor and LOH at D8S85 was associated with metastatic lymph nodes. The overall percentage of LOH in chromosome 9 was 54% and its highest incidence was for D9S126 (36%), followed by 26%, 21%, 19% for D9S166, D9S128 and D9S180, respectively. Fifty nine percent (59%) of the cases with LOH at 9q were diagnosed as squamous cell type (SCC), whereas 9% only were transitional cell type (TCC). No

significant association was recorded between the presence of schistosomiasis and LOH detected in all markers used in this study.

Conclusion: Our data indicate that more than one tumor suppressor gene on chromosomes 8 and 9 are involved in high grades of bladder carcinogenesis, one at 8p12 and another at 8q21.1 regions. Also, a region at 8q23-quaeter may harbor tumor suppressor gene that involved in metastasis of bladder cancer. Our study also revealed that 9p21 (p16^{INK4}) region is involved in both types of the tumor (SCC & TCC). PTCH located at 9q22.3, as well as the TSC gene at 9q34 are involved in squamous cell carcinoma rather than transitional carcinoma. Region 9q12-13 is considered to be a critical region of urothelial tumor suppressor genes. Finally, the present study shows no line of demarcation between schistosomiasis-associate and non schistosomiasis-associated bladder cancer in terms of LOH of the tested microsatellite markers on chromosome 8 and 9. This suggests that data obtained from schistosoma-associated bladder cancer can be extrapolated to bladder cancer induced by a schistosomiasis independent mechanism.

Key Words: Loss of heterozygosity (LOH) – Bladder cancer – Schistosomiasis – PCR.

INTRODUCTION

In Egypt, where schistosomiasis is hyperendemic, more than 20% of the population are infected with high rates of mortality and morbidity [1]. The Ancient Egyptians, settling and cultivating the Nile Valley, were among the first to contract schistosomiasis in an endemic manner. Thus, it was mentioned in medical papyri and in engravings on the walls of the temples as early as the sixth century BC [2], which was confirmed later by paleopathologic studies that demonstrated the eggs of the parasite in Egyptian mummies from the 20th dynasty [3]. One of the consequences of infestation with Schis-

tosoma haematobium is a marked increase in the incidence of bladder cancer. In Egypt, carcinoma of the bladder accounts for 20.6% of all tumors, constituting 31.7% of male and 5% of female cancers. At the National Cancer Institute, Cairo University, a higher total frequency was reported, namely 27%, according to the cancer pathology registry [4]. The peak age of diagnosis is usually 50±5 years with a male to female ratio of 5:1 [5]. This pattern in bladder cancer is in striking contrast with the situation experienced in western countries, where bladder cancer is the fifth to seventh most common cancer in men [6]. In Egypt, bladder cancer has a clinicopathologic pattern that differs in some important aspects from that seen in Europe and North America. Most tumors present as bulky fungating nodular masses with deep infiltration into the bladder wall (p3, 73%; p4, 16%), whereas papillary types are rare (7%). The majority of tumors are of the squamous cell variety, which represents 59% to 81% of cases in different reports. However, a recent trend towards a relative increase in the frequency of the transitional cell variety has been reported [7]. In western populations, the incidence of squamous cell carcinoma is between 3% and 6.7% of all bladder cancer cases [8]. Bladder carcinogenesis in Egypt is probably related to bacterial and human papilloma virus (HPV) infections associated with bilharzial infestation, rather than the parasite itself [9].

In the present days, the research is focusing on combining pathological variables with molecular markers in order to develop a comprehensive profile of the tumor with respect to their clinical outcome and response to therapy. These investigations have provided a wealth of knowledge to enhance our understanding of the genetic basis of carcinogenesis and genetic events that determine the clinical behavior and patient outcome.

The main purpose of this study is to investigate the role of chromosomes 8 and 9 in the initiation and/or progression of bilharzial-related bladder cancer through detecting the common regions of deletion using 8 different microsatellite markers and their association with different clinicopathological variables. It also aims to compare the LOH pattern of the tested markers between schistosomiasis-associated and non schistosomiasis-associated bladder cancer.

MATERIAL AND METHODS

Fresh tumor tissues and their corresponding blood samples (as controls) were obtained at the same time of surgical resection from 42 primary bladder cancer patients (schistosomal and non-schistosomal) who underwent total cystectomy at the National Cancer Institute, Cairo University. A portion of each tumor specimen was fixed in 10% formaline for histopathological examination and the presence of calcified schistosomal eggs in bladder tissues. Tumors were staged according to the TNM classification system [10] and graded according to WHO guidelines [11]. Table (1) shows the clinicopathological characteristics of the bladder samples obtained. High molecular weight DNA was extracted from tumor tissue and blood lymphocytes after digestion with proteinase K followed by phenol/chloroform extraction and precipitation using ethanol as described previously [12].

Table (1): Clinicopathological characteristics of studied cases.

| Clinico-pathological variables | Number of patients (%) (n=42) |
|----------------------------------|-------------------------------|
| <i>Schistosomal infestation:</i> | |
| Present | 27 (65%) |
| Absent | 15 (35%) |
| <i>Type of tumor:</i> | |
| Squamous | 29 (69%) |
| Transitional | 11 (26%) |
| Adenocarcinoma | 2 (5%) |
| <i>Histological grade:</i> | |
| 1 | 4 (9.5%) |
| 2 | 17 (40.5%) |
| 3 | 21 (50%) |
| <i>Tumor stage:</i> | |
| P3a | 10 (24%) |
| P3b | 26 (62%) |
| P4a | 4 (9%) |
| Unknown | 2 (5%) |
| <i>Nodal status:</i> | |
| Present | 18 (43%) |
| Absent | 21 (50%) |
| Unknown | 3 (7%) |

Loss of Heterozygosity (LOH) Analysis:

DNA samples extracted from tumor tissues and their corresponding peripheral blood were analyzed for allelic loss using 8 highly polymorphic dinucleotide microsatellite repeats distributed on different regions on chromosomes 8 and 9. The primer sequences for these markers were obtained from the Genome Database (<http://gdbwww.gdb.org>).

PCR amplifications were performed on 100ng of genomic DNA, 0.2 μ M concentration of primer, 200 μ M of each deoxy nucleotide triphosphate, 1 μ Ci of 32 P- α -dCTP (3000Ci/mMole), 1X reaction buffer with 1.5mM MgCl₂ and 1 unit of Taq DNA polymerase (Gibco, BRL, Germany). The reaction mixture was denatured at 95°C for 5min, then subjected to 35 cycles: for 1min at 94°C, 1min at the annealing temperature that varies according to the marker used, followed by 1min at 72°C, then final extension for 10min at 72°C. PCR products were electrophoresed, after denaturation at 95°C for 5min, on 6% polyacrylamide denaturing gel containing 7M urea at 50 Watts for 2-5 hours. The gel was dried and autoradiographed on X-ray film. The films were analyzed densitographically using BioDocAnalyze (Biometra, Germany) and the LOH was evaluated by comparing the intensity of alleles from normal and tumor DNA. The absence or significant decrease in one allele tumor was considered LOH.

Statistical Analysis:

Loss of heterozygosity at chromosomes 8 and 9 was correlated to each of the pathological parameters including schistosomiasis infestation, type of tumor, grade, stage, and lymph node status using the GraphPad prism computer system (GraphPad software, San Diego, USA). Fisher's exact test was used to test the correlation between the frequency of LOH and each of clinicopathological parameters, and the correlation was considered significant when *p* value was less than or equal to 0.05 (≤ 0.05).

RESULTS

Among the 42 tumors examined, 31 (74%) exhibited LOH for at least one marker on chromosome 8. The incidence of LOH for each marker was recorded, where the highest percentage (41%, 16/39) was observed in the D8S84 locus followed by D8S87 (37%, 13/35), D8S85 (29%, 11/38) and finally D8S88 (25%, 9/35). The overall percentage of LOH in chromosome 9 was about 54% (21/39) and the incidence for each individual marker was 36% for D9S126, 26% for D9S166, 21% for D9S128 and finally 19% for D9S180. Fig. (1) shows representative samples for LOH on different markers at chromosomes 8 and 9.

The Association between LOH and the Histological Type of the Tumor:

The frequency of LOH (both chromosomes 8 & 9) was 83% (24/29), 73% (8/11), and 100% (2/2) for SCC, TCC, and adenocarcinoma, respectively. The frequency of LOH at chromosome 8 alone was recorded as 76% (22/29) for SCC, 64% (7/11) for TCC and 50% (1/2) for adenocarcinoma. LOH on the short arm of chromosome 8 markers was 43% (9/21) for SCC, 67% (4/6) for TCC and 0% (0/2) for adenocarcinoma. The LOH observed at 8q markers was 62%, 45%, and 50% for SCC, TCC and adenocarcinoma, respectively. LOH at the individual markers on chromosome 8 showed an insignificant correlation with the histological type of the tumor. The frequency of LOH on chromosome 9 markers was 16/27 (59%) for SCC, 5/11 (45%) for TCC, and 2/2 (100%) for adenocarcinoma. Thirty three percent (33%) (7/21) of LOH on the short arm of chromosome 9 were classified as SCC, 44% (4/9) were TCC, and 50% (1/2) were adenocarcinoma. However, 59% (16/27) of LOH detected on chromosome 9q were of the squamous cell type, 9% (1/11) only were of the transitional type, and 100% (2/2) were adenocarcinoma. This association was insignificant ($p=0.160$). The results for individual markers showed an insignificant correlation with the histological types of the tumor.

The Association between LOH and Schistosomiasis:

Out of 42 cases examined only 27 were associated with schistosomiasis, 22 of these 27 cases (81%) revealed LOH in at least one marker used for both chromosomes (8&9). Thirteen of the 15 (87%) cases that were free of schistosomiasis showed allelic losses. LOH detected at chromosome 8 showed that 67% (18/27) were associated with schistosomal infestation and 80% (12/15) were non-schistosomal. Thirty and 54% of LOH were detected in schistosomal and non-schistosomal cancer on the short arm of chromosome 8 (8p), whereas 56% and 76% of LOH were on the long arm of the chromosome, respectively. The incidence of LOH for individual markers on chromosome 8 with schistosomiasis was calculated as 42%, 37.5%, 24% and 3% for D8S84, D8S85, D8S88, and D8S87 respectively, however, this association was statistically insignificant.

Sixty-eight percent (68%) of the cases diagnosed as schistosomal bladder cancer had LOH on chromosome 9, whereas of 14 non bilharzial BC, 8 (57%) had LOH. Results of LOH for each chromosomal arm showed 30% of bilharzial bladder cancer (BBC) (6/20) had LOH and 50% of LOH (10/20) were detected in non-

bilharzial bladder cancer (NBBC) on the short arm of chromosome 9. LOH on 9q showed that 14% were BBC and 40% were NBBC. The individual markers on chromosome 9 showed almost the same percentage of LOH for BBC cases; 27% for D9180, 29% for both D9S166 and D9S158, and 30% for D9S126.

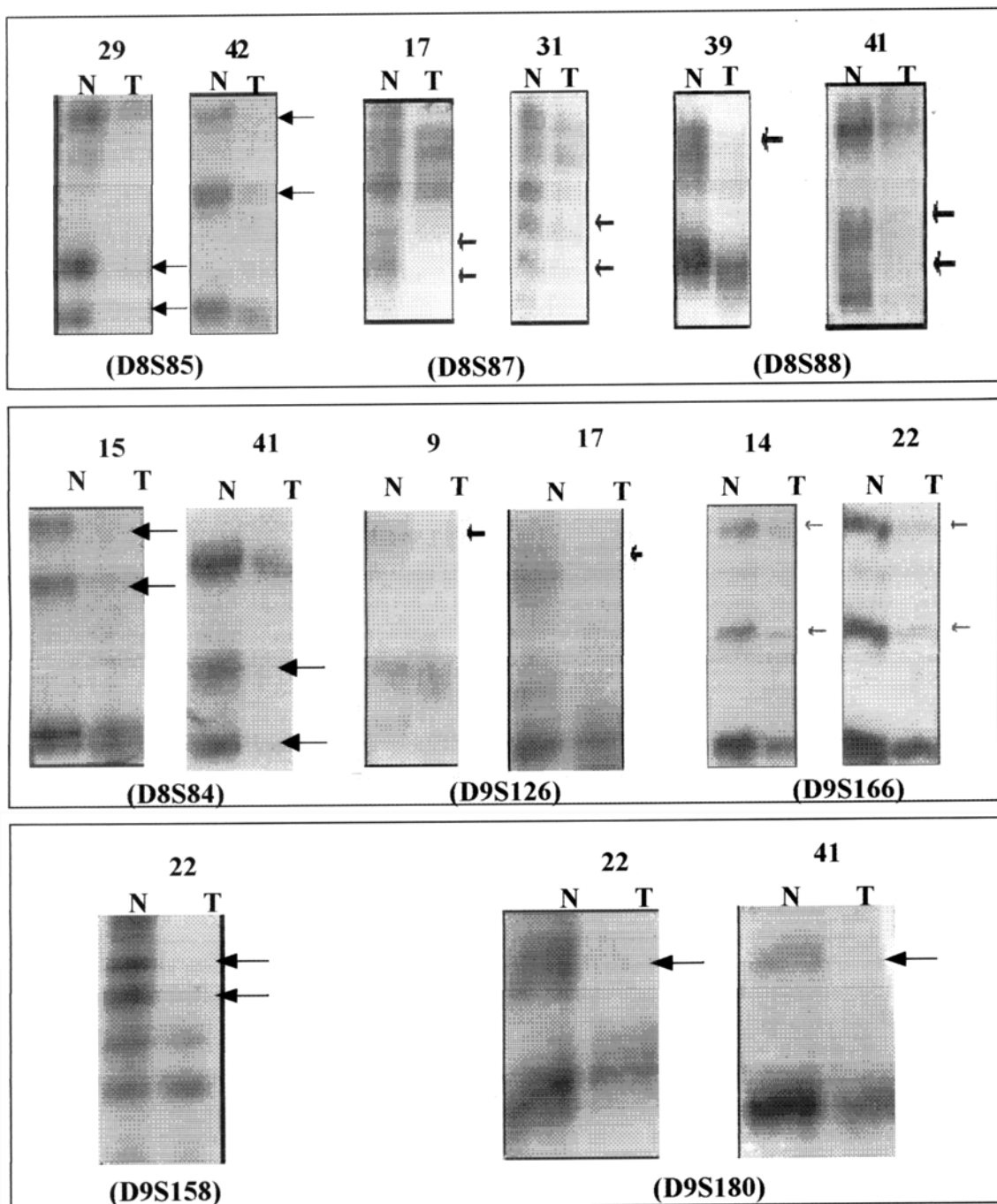


Fig. (1): Loss of heterozygosity on different markers at chromosomes 8 and 9. N, the normal; T, the tumor and the arrows indicate the deletion of one allele.

The Association of LOH with Tumor Grade:

Frequency of LOH on both chromosomes (8&9) were 75% (3/4), 77% (13/17), 90% (19/21) for G1, G2, and G3, respectively. The incidence of LOH on chromosome 8 showed that 2 of 4 cases were G1 (50%), 13/17 (76%) were G2 and 15/21 (71%) were G3. It was observed that LOH in each chromosomal arm increased proportionately with the histological grades, for example at 8p the LOH was 0%, 33% and 50% for G1, G2, and G3, respectively. The percentage of LOH at 8q was 50%, 53%, and 62% for G1, G2, and G3, respectively and the association was an insignificant ($p=0.5495$ and 0.9467 for 8p and 8q respectively). The data of LOH for each individual locus used on chromosome 8 showed an insignificant association with tumor grades where p value was 0.5495, 0.6013, 0.9964, and 0.6419 for D8S87, D8S84, D8S85 and D8S88 respectively. The incidence of LOH on chromosome 9 occurred in 75% (3/4), 64% (9/14) and 58% (11/19) of G1, G2, and G3 tumors, respectively. LOH for each chromosomal arm as 25% (1/4), 33% (4/12), and 44% (7/16) for G1, G2, and G3, respectively on the short arm of the chromosome. On the other hand, at the long arm (9q), the incidence of LOH recorded was 75% (3/4), 43% (6/14), and 39% (7/18) for histological G1, 2, and 3, respectively. Data of the LOH for each individual locus used on chromosome 9 showed an insignificant correlation with tumor grade.

The Association of LOH with Staging of the Tumor:

The percentage of LOH on chromosome 8 and 9 increased with the progress of staging where it was 62.5%, 68%, and 100% for p3a, p3b, and p4a, respectively. The LOH recorded at chromosome 8 alone was 62.5% (5/8), 100% (4/4) and 68% (17/25) for p3a, p3b and p4a, respectively, whereas it was 33%, 39% and 50% at the short arm of chromosome and 62.5%, 52% and 75% at the long arm. An insignificant association was observed between LOH for individual markers of chromosome 8 with stage of the tumor. LOH recorded at chromosome 9 was 25% (2/8), 29% (6/21) and 25% (2/4) for p3a, p3b and p4a, respectively whereas it was 17%, 48%, 0% at the short arm of chromosome and 14%, 57%, 50% at the long term. An insignificant association was observed between LOH

for individual markers of chromosome 9 with stage of the tumor.

The Association of LOH with Lymph Node Status:

The association between deletion with the metastatic status of the tumor was indicated in this study by the presence of tumor cells in lymph node. Statistical significance ($p=0.0281$) was found between LOH on chromosome 8 and lymph node status, where 89% who had LOH were positive while 57% who had LOH were negative for lymph node involvement. An insignificant association was observed for LOH on either chromosomal arms or individual markers on chromosome 8 with lymph node status. Fifty percent of LOH on chromosome 9 detected were shown to be associated with positive lymph nodes, while 72% were negative for lymph node. This association is still insignificant ($p=0.5149$). Meanwhile, the association between LOH on either chromosomal arms or individual markers with lymph node positivity was statistically insignificant.

DISCUSSION

Two distinctly different bladder cancers exist in Egypt, namely schistosomal and non schistosomal types. The less common non schistosomal type is observed among the urban population, and affects elderly patients who usually present with superficial tumors and the histopathology is invariably of the transitional cell type. The schistosomal type is prevalent in rural areas. The peak of incidence occurs earlier in life, during the 4th and 5th decades [13]. The majority of tumors are of the squamous cell type, however, a recent trend towards a relative increase in the frequency of the transitional cell variety has been reported [7]. Detailed molecular genetic studies of the transitional type of cancer bladder have led to a working hypothesis of tumorigenesis and progression and to the use of some of these markers as determinants of prognosis and predictors of the ultimate clinical outcome [14]. Similar studies in schistosomiasis-related bladder cancer are limited. The present study aimed to gain insight in locations and frequencies of regional chromosomal aberrations in both chromosomes 8 and 9 in schistosomiasis related bladder cancer in order to clarify the role of these chromosomal regions in the development and/or progression of this type of tumor and to correlate those chromosomal changes

with different clinico-pathological criteria, as well as to non schistosomiasis-related bladder cancer.

Chromosome 8 and Bladder Cancer:

The short arm of chromosome 8 is frequently lost in human cancers. Loss of heterozygosity of this region has been reported in many types of cancer tested, including prostatic [15,16], colorectal [17], lung [18], hepatocellular carcinoma [19], bladder [20], oral and laryngeal squamous cell [21] and breast [22]. In the present study, chromosomal deletion on 8p was represented by D8S87 microsatellite marker located at 8p12. LOH was detected in 37% of the tumor samples examined. This frequent deletion suggested that the tumor suppressor gene at this region could be involved in the development of bladder cancer in Egypt. It was previously reported that a candidate tumor suppressor gene located between the loci D8S87, D8S283 and D8S133 at 8p12-21 region was involved in cancer progression [23]. A fine deletion mapping for chromosome 8 denoted two distant regions of deletion, i.e. a 10 cM telomeric region was located at 8p22 and a 17 cM centromeric region was located at 8p11.2-21.1 and the distance between telomeric and centromeric regions of common deletion was 3 cM and was involved in bladder cancer incidence [20].

Mapping of the same region showed that two genes, mainly the DNA polymerase beta gene (Po1B) and the subunit of protein phosphates 2A may play a regulatory role in many cellular pathways [24]. In this respect, putative candidate genes such as the FEZ1/LZTS1 gene, encoding a leucine-zipper protein, the Frizzles-related FRP1/FR2B gene, the death receptor genes as mediators in p53-dependent apoptosis and a gene, DBC2, of hitherto unknown function, all mapped within the 8p12-22 region [25-27].

In the present study, LOH recorded at D8S87 was frequent in both types of bladder tumor, namely SCC and TCC. The same results were obtained for either lymph node status, schistosomal bladder tumor type, which may indicate that the tumor suppressor gene located at 8p12 region could be involved in bladder carcinogenesis irrespective of the tumor origin and its aggressiveness. Data of the present study revealed that LOH occurred in high grades (II

and III) but not deleted in grade I, indicating that the gene(s) located at this region might be involved in high grades, a finding reported by previous studies [15,28] which detected deletions at 8p in high grade and stage of tumors. The results of this study also indicated that LOH on chromosome 8p represent a relatively frequent, non random genetic change in bladder cancer patients.

Gain of sequences on chromosome 8 was reported in different types of cancer and it was found to be associated with high tumor grade and stage [29,30]. The target gene for this gain is currently not known. Chromosome 8q24 harbors the MYC oncogene. Amplification of this region was detected in a subset of metastatic and recurrent tumors [31] and was shown to correlate with the presence of regional lymph node metastasis [32] as well as with poor prognosis [16,33]. Furthermore, gain of EIF353 located at distal 8q and encoding a eukaryotic translation initiation factors; was found to be associated with high-grade and high-stage prostatic cancer [34]. Another candidate gene in this region may be the prostate stem cell antigen, mapping to 8q24.2, whose overexpression is correlated with grade, stage and androgen-independence [35]. Additional gain of genes located on chromosome 8q has been detected in bladder tumors using comparative genomic hybridization (CGH) [36,37]. In the present study, 50% of the cases examined at 8q showed LOH in at least one of the 3 markers examined and the insignificant association observed with either of the different pathological factors may indicate that tumor suppressor gene(s) is/are located at 8q and is involved in bladder cancer irrespective of their type, grade, stage, and lymph node metastasis. In addition, no role of schistosomiasis was recorded in the 8q region.

The data of the present study for marker D8S84 which is located at 8q 21.1 indicated that the percentage of deletion increased from grade I, II, to III, indicating that the tumor suppressor gene located at this site may be involved in high grade tumors of the bladder. At the marker D8S85, LOH observed was associated with tumor positive lymph nodes rather than negative ones, which may predict that tumor suppressor gene may work as a metastatic tumor suppressor gene in this particular type of tumor. Most of the tumors investigated in

this study were of high grade and stage. The limitation of the patient numbers in each group led to a statistically insignificant results that were observed between LOH of markers and different clinicopathological criteria. Additional studies are needed to evaluate these regions as an early event in bladder carcinogenesis.

Chromosome 9 and Bladder Cancer:

Loss of heterozygosity on chromosome 9 is the most frequent genetic alteration identified in bladder tumors and is present in all stages and grades, suggesting that loss of one or more suppressor genes on chromosome 9 may be an early event in bladder tumorigenesis [38,39]. Data of the present study showed high frequency of deletion on both arms of chromosome 9, which indicates that they harbor more than one tumor suppressor gene involved in schistosomiasis-related bladder carcinogenesis. At 9p21 region, as indicated by D9S126 marker, LOH recorded was 30% and 50% in both schistosomal and non schistosomal bladder cancers. The same results were obtained when we compared LOH in squamous and transitional cell types, where both exhibited LOH at 9p21 region. This observation indicated that 9p21 region harbors a tumor suppressor gene involved in bladder cancer irrespective of the presence of schistosomiasis or the tumor type. Previous reports identified deletion on 9p21 region in different types of cancer, including schistosomal bladder cancer [7,40-42]. The p16^{INK4A} tumor suppressor gene, which is a negative regulator of cyclin-dependent kinase 4 is localized within this region [43]. Another tumor suppressor gene, p15^{INK4B}, has been identified in this region and deletions occur frequently in cancer cell lines and certain malignancies [44,45]. Previous studies on schistosomal bladder cancer showed high frequencies of LOH at 9p21 region [46]. P16^{INK4A} is inactivated by homozygous deletion in many transitional cell cases [47] and in 53% of the schistosomal related bladder tumors [48]. Also, p16 homozygous deletions were observed at high frequency in both schistosomal and non-schistosomal bladder tumors [49]. In this study, LOH at 9p21 region was detected in all grades and stages, which confirms previous studies that considered p16^{INK4A} gene as an early event in bladder carcinogenesis [40,42,50].

In this study, the markers selected covered three common deletion regions, at 9q. They are

D9S158 at 9q 34.3, D9S166 at 9q 12-21 and finally D9S180 at 2q22.3. High frequency of LOH at 9q arm and the individual markers on that arm were observed in squamous cell types rather than transitional, which indicated that 9q might be involved in carcinogenesis of this type of tumor. Human PTCH gene has been cloned, sequenced and found to be within the candidate region for basal cell nevus syndrome (BCNS), also known as Gorlin syndrome [51,52]. The PTCH gene is located at the 9q22.3 region, between D9S12 and D9S180 markers, and has been suggested as a tumor suppressor gene in basal cell carcinoma [53]. It was shown that familial and sporadic basal cell carcinoma of the skin display a loss of heterozygosity and mutations throughout the entire gene [52,54]. The present data revealed LOH in both schistosomal and non-schistosomal bladder cancers. Meanwhile, squamous cell type showed LOH at this region with frequency of about 22%, whereas none of the 11 cases of the transitional type had LOH indicating the involvement of that gene in squamous cell carcinoma rather than the transitional type. Detection of LOH at PTCH gene in all grades and stages may indicate its participation as an early event in bladder cancer incidence.

TSC is the gene for the autosomal dominant disease Tuberous Sclerosis which is characterized by the development of hamartomatous growths in multiple organ systems [55]. The gene TSC-1 has been demonstrated to be silenced in some TSC hamartomas and so is hypothesized to act as a tumor suppressor gene [56,57]. The gene is located at 9q34 region. It was reported that TSC is involved in renal [30], and ovarian tumors [58]. Furthermore, mutations and LOH were reported for TSC gene in bladder tumors [59]. Our data showed that region 9q34.3 exhibited LOH in schistosomal and non-schistosomal bladder cancers, but skewed towards the schistosomal type. Twenty-two percent (22%) of the squamous cell type harbor deletions at that region, whereas none of 11 transitional cell types showed retention of alleles which may indicate, like PTCH gene, that TSC is involved in squamous cell carcinoma rather than the transitional type. A previous study suggested the TSC gene as a bladder cancer suppressor gene and is associated with recurrence of superficial bladder cancer [36].

Data obtained from the present study using D9S166 marker located at 9q12-21 showed LOH in both types in bladder cancer (SCC & TCC), while the role of schistosomiasis is not clear. High frequency of LOH was detected in high tumor grade which indicated that this region is involved in high grade tumors. It was proposed that q12-13 region may act as the site of the critical tumor suppressor gene or genes involved in early urothelial neoplasia [36,60].

These observations suggest the following: First, deletions of genes on 9q including PTCH and TSC genes may be early events in squamous cell carcinoma of bladder development irrespective of schistosomal status of the tumor. Second, 9p21 region may be involved in both tumors (SCC & TCC). Third, the region of 9q12-13 is considered to be a critical region for tumor suppressor genes in bladder cancer. Fourth, there are more than one tumor suppressor gene at chromosome 8 involved in bladder carcinogenesis, including 8p12 and 8q21.1 which may be involved in high grades. Finally, 8q harbors tumor suppressor genes involved in metastatic tumors of the bladder.

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