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Detection *P53, Rb1* and *H-ras* Loss of Heterozygosity LOH in Patients with Urinary Bladder carcinoma

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Abstract

In this study 22 samples of urine sediments were obtained from patients with Urinary Bladder Carcinoma, DNA base technique and LOH detection was investigated in three genes *P53*, Rb1 and *H-ras*. The result are shown that LOH was present in most cases and for all genes where LOH in *P53* represents (31.8%) and for *Rb1* (45.4%) and for *H-ras* gene (27.2%).In some cases, more than one gene locus mutations were found.

Introduction:

Bladder cancer is the second most common malignancy affecting the urinary system. Approximately 90% of urothelial tumours are urothelial cancers (UCs). The remaining tumours are squamous cell cancers or adenocarcinomas. Small-cell carcinomas account for less than 1%. The spectrum of bladder cancer includes non–muscle-invasive, muscle-invasive, and metastatic disease, each with its own specific typical behaviour, prognosis, and treatment (Vrooman O.& Witjes j.2008).

Bladder cancer is one of the most common cancers in human. Almost there are two major types. Approximately 70% of bladder cancer patients present with non-muscle invasive (NMI) tumors, which recur frequently and are usually not fatal but make bladder cancer one of the most expensive cancers to treat . The remaining 30% of patients present with muscle-invasive (MI) tumors.(Jin X.*et al* 2014 ; Dancika G. *et al* 2013)

DNA-Based techniques reflect more strongly invasiveness and prognosis of the cancer and these techniques may be used as a good addunctive tests for evaluation of the bladder cancer.(Zachary L.*et al* 2013; Wang P.*et al* 2014) .

LOH at the region of a known tumor suppressor gene is considered indicative of a mutation in the remaining copy of the gene, resulting in the loss of the tumor suppressing function of the gene product. Several studies reported frequent alterations of chromosome 9, occurring early tumorigenesis, but also present at all stages and grades (Ploussard G .*et al* 2010).

The detection of Loss of Heterozygosity (LOH) for some genes associated with tumors was highly investigated and LOH was considered as the most frequent mutation of some genes such as *P53*, *Rb1* &*H-ras* , which arise from relatively large deletions particularly in the exons(Innis M. *et al* 1990).

Loss of heterozygosity (LOH) has been described as a distinct and frequent type of molecular alterations. LOH at the region of a known tumor suppressor gene is considered indicative of a mutation in the remaining copy of the gene, resulting in the loss of the tumor suppressing function of the gene product.
(Burger M..*et al* 2006 , Berger A.*et al* 2002).

On the other hand *P53, Rb1* and *H-ras* genes are tumor suppressor genes and are considered very essential in control and regulation of other genes particularly oncogene, *RAS* mutations common in non-muscle invasive (NMI) tumors and *P53/RB1* impairment common in muscle-invasive(MI)tumors (Dancika G. *et al* 2013).

The aim of this study is to detect of LOH in patients with Urinary Bladder Cancer by using three genetic markers for *P53 , Rb1* & *H-ras*.

Material and Methods

Patients: urine sediments are obtained from 24 patients with urinary bladder carcinoma.

DNA extraction: DNA extraction from leukocyte fractions, the urine sediments was done by Sacace DNA extraction kit, using manufacturer's protocol.

Primers: DNA amplified with three primers.

p53 (F)	5 – GAT GCT GTC CGC GGA CGA TAT -3
p53(R)	5 – CGT GCA AGT CAC AGA CTT GGC -3
Rb1(F)	5- TT CAA TGA AGA ACA AAT GG -3
Rb1(R)	5- GCA ATT GCA CAA TCC AAG TT – 3
H-ras(F)	5-GAC-GGA-ATA-TAA-GGC-TTG-TTG-3
H-ras(R)	5- TGG-ATG-GTC-AGC-GCA-CTC-TT-3

Samples were amplified in the manufacturer's reaction buffer containing 20 pmol of each primer, 0.2 mM of each dNTP, 2 mM MgCl2, and 2 U Taq DNA polymerase(promega) Amplification was carried out for *p53*(94◦ C for 2 minute), of 40 s at 95◦ C , 35s at 58 ◦C & 35 C at 72 C at 40 cycle minutes. *Rb1* 1 minute at 94◦ C, 1 minute at 50◦ C and 1 minute at 72◦ C for 30 cycles.*H-ras* 94C◦ for 5 min, 94C◦ for 15 Second, at 65 C◦ for 30 Second at 35 cycles.

Restriction fragment length polymorphism (RFLP*) analysis.*

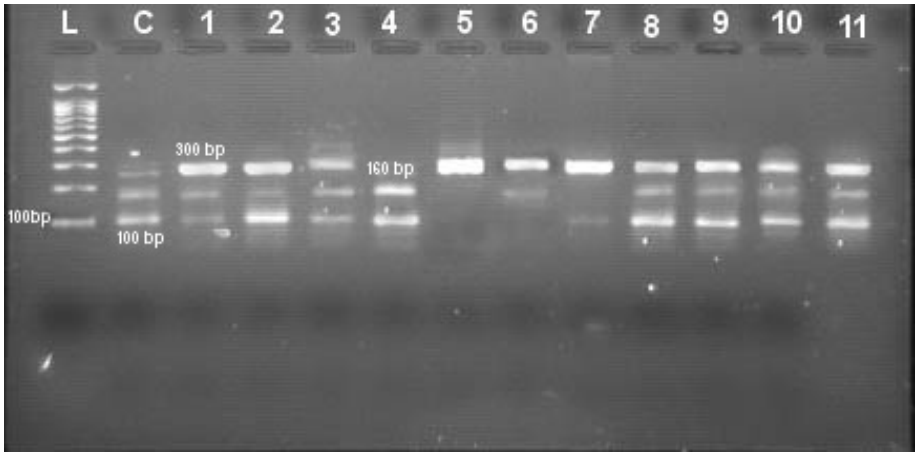
After amplification, 25 ul. of PCR products was digested with 2 to 20 units of the appropriate restriction enzyme(Bst UI , Xba1 & Msp1) for p53 ,Rb1 &H-ras respectively over night.. Each product was run on an agarose , which was stained with ethidium bromide and photographed under ultraviolet light. Loss of heterozygosity(LOH) was defined as a visible change of **1** allele in the tumor **DNA** compared with normal DNA. When normal DNA did not show 2 different alleles (that is, homozygous), the case was considered uninformative.

Discussion:

Loss of Heterozygosity (LOH):

LOH studied by using DNA extraction from urine sediment for patients with urinary bladder carcinoma & the main markers used in this study is *P53* , *Rb1* and *H-Ras* genes.

Identification of DNA mutations in urine sediments has been proposed as a noninvasive and early indicator of urinary tract cancer.



Figure(1) *P53* gene , Bst UI digestion PCR product of the urine sediement DNA , L= DNA size marker ; C= Control; 1,2,3,4,8, 9,10,11 =heterozygous patients 5,6,7= homozygous who are uniformative for LOH

Total of (22) Patients subjected for this study who have Urinary Bladder carcinoma, it was shown that 7(31.8%) patients was positive for LOH as shown in Figure (1).

The result have suggested that detection of LOH of *P53* gene by PCR-RFLP may be a good tool for evaluation of bladder cancer and according to the data mentioned by (Borkowska *et al* , 2007).

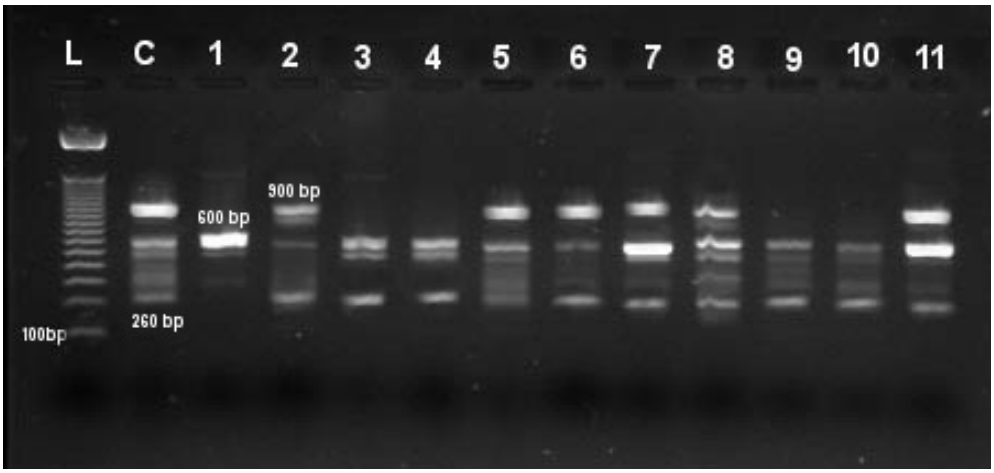
In healthy controls the lymphocyte and urine DNA displayed the same electrophoretic patterns, which indicated absence of microsatellite alterations. (Sourninos G.*et al* 2001)

It is considered the present of one band or two band are uninformative and homozygous whereas the presence of three band are informative and heterozygous also are natural in the genome.

Many studies have confirmed that *P53* has a role in Urinary Bladder Carcinoma when exposed to mutation (Berggren *et al*, 2001).And also in colon cancer & prostate cancer (Spajic B. *et al.*2005).

These studies revealed that *P53* gene mutation are usually seen to occur as late events in Bladder tumorigenesis & also it was showed that LOH at *P53* loci is higher significant and being associated with tumor grade& stages (Telu K.*et al* 2013).

LOH studies suggest that deletions of the *p53* gene occur frequently during the development and progression of urotherial carcinoma of the urinary bladder. (Cheng L.*et al* 2004)
The result of this study are correlated with that obtained by Hiroshi M. *et al* 1996,who found 38% showed LOH at one or more loci.
Although *P53* gene was proved its role in Bladder tumorigenesis but in the present study have showed that only (31.8%) of patients give positive result for LOH as a result of *P53* mutation &the result are normal for this gene this result suggest that P53 mutation may occur at the last grade &stages not that early stages as mentioned by (Borkowska *et al* , 2007).



Figure(2): *Rb1* gene , Xba1 digestion PCR product of the urine DNA, L= DNA size marker C= Control 2,5,6,7,8,11=heterozygous patients 1,3,4,9,10 = homozygous who are uniformative for LOH

About 10 of 22 patients(45.4%) uninformative cases showed LOH at the *Rb1*.*Rb1* is as *P53* is tumor suppressor gene which involved in pathogenesis & progression of Urinary Bladder carcinoma. LOH of *Rb1* gene has frequently be seen in Retinoblastoma, Bladder, Prostate, Breast & Lung carcinoma.
The result of this study as correlated with that (Acikbas I.*et al* 2002) that over 40% of Bladder cancer have positive *Rb1* LOH and also with that (Traczyk M. *et al* 2011) who found that 34% of Urinary bladder carcinoma were positive for *Rb1* LOH and this frequency is lower (12.5%)than reported in some researchers (Gallucci M.*et al* 2005).
According to positive data obtained in this study, It was shown that *Rb1* is mostly seen in cases negative to *P53* ,except three cases which have positive to *P53* this suggests that *Rb1* LOH occur mostly in early stages of cancer and mainly in tissues with low grade as mentioned by (Traczyk M. *et al* 2011; Wada T.*et al* 2000).Besides, the present study is correlated with that stated by (Philips S. 1994), who observed that most cases of *Rb1* LOH is carried out in the early stage of tumorigensis in the bladder. Some studies revealed the presence of co existent abnormalities of multiple tumor suppressor gene and also suggested cooperative roles for both *P53* &*Rb1* genes, Here it was observed two loci for the cases that positive simultaneously for *Rb1* LOH & *P53* LOH. This result suggests that the cases reach high grades & stages this result identical to that obtained by (Miyamoto H. *et al* 1996).

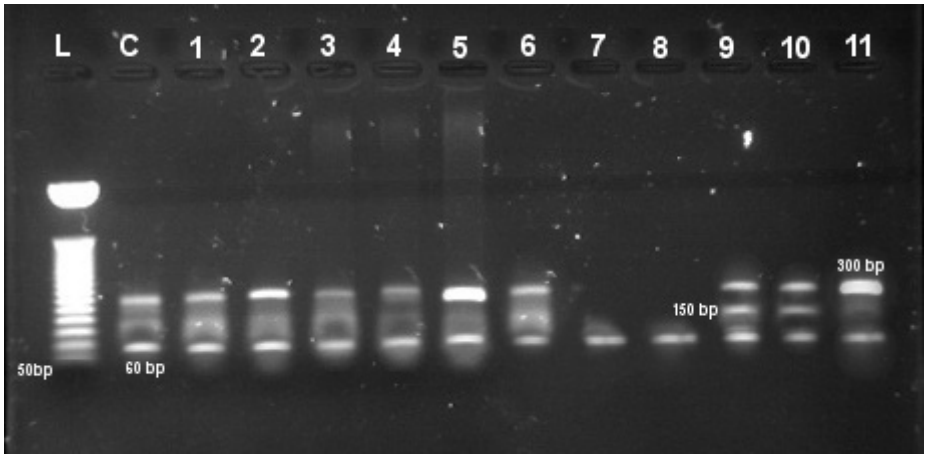


Figure (3) *H-ras* gene ,MspI digestion PCR product of urine sediment DNA ,L=DNA size marker; C= control 1,2,3,4,5,6,9,10,11= heterozygous patients 7, 8 =homozygous patients who are uniformative for LOH

In addition to *P53*, *Rb1* LOH, *H-ras* LOH is also invistigated , it was seen that only 6 (27.2%)patients gave positive for *H-ras* LOH ,whereas the others are normal for this gene (figure3).

H-ras gene is Known has a role in many cancers such as lung, bladder, breast& skin and this gene is considered as pro- Oncogene (Kratz C.*et al* 2007).

LOH of *H-ras* in human tumor may indicate that this gene has a tumor suppressor role, since normal *H-ras* can suppress the malignant phenotype of transformed cell lines, acting as a tumor suppressor gene (Gorringe K. *et al* 2009).

The present study also showed that *H-ras* LOH was simultaneously associated with *P53* &*Rb1* in some cases and it is known that there is no correlation between tumor grade and stages with *H-ras* mutation. But *Ras* mutations common in NMI tumors and *P53/RB1* impairment common in MI tumors (Dancika G. *et al* 2013).

The *Ras* genes are the most common mutations found in Bladder cancer and up to 13% of all bladder tumours harbour a mutation in *H-ras, K-ras* or *N-ras*. There is limited data available regarding these oncogenic mutations in BC of patients <20 years. (Beukers W. *et al* 2013)

But *H-ras* gene was found to associate with other cancer cells particullay in Prostate, neck & head and so (Kiaris H.*et al* 1994).

Patients who exhibited microsatellite alterations (LOH) in urine specimens exclusively had early, moderate or late stages and grade I, II or III disease.

Briefly prospective analysis of bladder tumors involving each of these genetic loci implicate *P53* inactivation as a late events in progression that is associated with the transition of tumor from a low grade to a high grade lesion(Sidransky D.*et al* 1991, Fujimoto K.*et al* 1992 , Sarkis A.*et al* 1993).

Loss of expression of *Rb1* gene product is associated with the invasive phenotype, and both the *P53* and *Rb1* genes have been proposed as indpendent prognostic indicators of progression in Bladder cancer (Cardo C.*et al* 1992)

Some studies have shown an association of the *H-ras* mutations with low-grade, noninvasive superficial papillary urothelial tumors, while others have suggested that the mutations play a role in bladder tumor invasion (Mo L. *et al* 2007); and also Jebar A.*et al* 2005 found no correlation between *H-ras* mutations and tumor progression .

Finely the detection of loss of heterozygosity in cytological urine specimens may be prognostic indicator of early detection of bladder cancer.

Many studies have examined only *H-ras* and have reported a wide range of mutation frequencies (0- 70%) that may reflect true differences in the tumors examined or technical differences between assays. Currently, there is agreement from several studies that the frequency for *H-ras* is in the range of 10-20 % (Pandith A. *et al* 2010).

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