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P53 and GSTs Polymorphisms among Bladder Cancer Patients in Iraq

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Abstract:

The role of metabolic genes for glutathione S-transferase (gstm1, gstt1) and P53 exon-7 and 8 as risk factors for bladder cancer in Iraqi patients was studied. Thirty five samples from patients attended the Nuclear Medicine Hospital in Baghdad plus fifty samples from apparently healthy individuals were included in the study.

DNA extraction was performed using proteinase K/ SDS method PCR techniques were followed to amplify the gene using specific primers for detection of gstm1, gstt1 and albumin as internal control. Single strand conformation polymorphism (PCR-SSCP techniques) for detection of point mutation in P53 exon-7 and 8 were used.

Genetic analysis showed that gstm1 null genotype was statistically associated with bladder cancer (14.29%), this association was strengthened when both gstm1 and gstt1 alleles were absent (28.5%). For detection of the point mutations in P53 exon-7 and 8 Single Strand Conformation Polymorphism (PCR-SSCP) have revealed that there was high association between exon-7 mutations and incidence of bladder cancer (42.86%), while the association between exon-8 mutations and bladder cancer was low (14.28%). In case of combination between exon-7 and 8 mutations, the association was also low (11.42%). Mutations in gstm1 and exon-7 being associated together are more than with exon-8 mutations. The number of mutations in P53 gene was more in patients with combined genotype of gstm1 deletion and null genotypes.

Keywords:

gstm1 and gstt1, P53, ,Bladder cancer

Introduction:

Glutathione S-transferases (GSTs) are a super gene family of inducible enzymes important in the metabolism of many different xenobiotics in mammals, including environmental carcinogens, reactive oxygen species and chemotherapeutic agents [1]. They act as phase II metabolizing enzymes, catalyzing reaction between glutathione and various electrophilic compounds resulting in less reactive and more easily excreted glutathione conjugation [2].

In human, this super gene family is divided, based on chromosomal location and sequence homology into four classes, termed alpha α (gsta), Mu (gstm1), Pi (gstp) and theta θ (gstt1). These classes differ in their tissue specific expression and distribution within tissue [3].

The gstm1 gene, codes for the cytosolic enzyme, has received considerable attention in relation to bladder cancer and other smoking-related cancer because of its role in the detoxification of benzo [a] pyrene and other polycyclic aromatic hydrocarbons (PAHs) found in tobacco smoke by conjugating them with glutathione [4]. Many studies have indicated that the gstm1 null genotype is associated with an increased risk of lung, bladder and colon cancers [5,6,7,8]. Others [7] noted that the null genotype was associated with a two-fold increase in breast cancer risk, primarily among postmenopausal women. In contrast to gstm1, studies exploring the potential role of gstt1 genotype in individual susceptibility to bladder cancer have yielded inconsistent results. Few studies showed decreased risk of bladder cancer with gstt1 null genotype [4,8] whereas others showed increased bladder cancer risk with gstt1 null genotype [9,10]. Inconsistent results have also emerged from the other studies exploring the potential combined effect of gstm1 and gstt1 genotypes in development of this malignancy [6,10].

The fundamental position of P53 as the guardian of the genome reflects its central role in the DNA damage response [11]. The P53 gene is the most frequently mutated in human cancers. The P53 protein has several biological functions such as involvement in cell cycle regulation, programmed cell death, senscence, differentiation and development transcription DNA replication initiation complex, in addition, P53 could have the passage from the G1 phase and the S phase of the cell cycle (12). DNA repair and maintenance of genomic stability (13).

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Cells defective for P53 are unable to arrest in G1 phase in response to Ultra Violet Light and show reduced PCD (Program Cell Death), [14]. The P53 tumor suppressor protein plays a key role in the regulation of the cell cycle and cell death and involved in cell differentiation, DNA repair, senescence, and angiogenesis. P53 activates or inhibits transcription by binding to specific DNA target sequences [15]. Alteration in P53 gene and/ or its protein product have been linked to the development of many lesions of cancer including skin, esophagus, testes, breast, and bladder cancer.

We present here the association pattern of gstm1 null genotype with bladder cancer and the combined effect of gstm1 and gstt1 genotypes in development of this malignancy. We also present the association between exon 7 and 8 mutations of P53 and bladder cancer in Iraq.

Materials and Methods:

Sampling

Ninety patients with bladder cancer attended the Nuclear Medicine Hospital in Baghdad for treatment were interviewed and asked to fill up medical and demographical questionnaires. The criteria included age, Schistosoma infection, occupation, family history and smoking behavior. Stage, grade and type of cancer were obtained. However, many of the patients declined later to donate samples. Other technical problems associated with sampling and preservation of DNA have reduced the number of samples to only 35 (26 males and nine females). Also 50 apparently healthy individuals of comparable ages but with no history of cancer or bladder diseases included in the study.

GST Amplification

The polymorphism of gstm1 and gstt1 were analyzed by a multiplex PCR procedure [16]. The following primers were used :

GSTM1:
F-(5/ - GAA CTC CCT GAA AAG CTA AAG C)
R-(5/ - GTT GGG CTC AAA TAT ACG GTG G)
GSTT1:
F-(5/ - TTC CTT ACT GGT CCT CAC ATC TC)
R-(5/ - TCA CCG GAT CAT GGC CAG CA)
Albumin:
F-(5/ - GCC CTC TGC TAA CAA GTC CTA C)
R-(5/-GCC CTA AAA AGA AAA TCG CCA ATC)

The amplification reactions were carried out in a volume of 50 µl containing (25ng) DNA;10 mM Tris–HCl; 50 mM KCl ; 1.5 mM MgCl2;200 µM (each) dATP, dCTP,dGTP and dTTP (Promega); each primer was at 20 pM and 2.5 unit of Taq polymerase (Promega) . The amplification was carried out as initial denaturation at 95 oC for 3 min, 30 cycles in thermocycler (Techne,Cambridge Ltd., England) as follows : 94 oC for1 min; 59 oC for1 min ; 72 oC for1 min and 5 min final extension for last cycle. The PCR products were analyzed on 2%Agarose gel electrophoresis to detect the absence or presences of these genes. Albumin gene was used as internal control. The amplified Albumin fragment was 350 bp in length, whereas presence of the gstm 1 and gstt1 genes were identified by 215 and 480 bp fragments, respectively.

PCR-Single Strand Conformation Polymorphism (SSCP) for P53 gene (Exones-7 and 8) mutations

Gene mutations of P53 were analyzed by PCR-SSCP according to the protocol of Vega [14]. The amplification was carried out on a thermocycler (minimcy Mj Research). Genomic DNA was amplified by using four sets of primers (Alpha DNA ,Canada).

Exon-7
F-(5¯-AAG GCG CAG TGG CCT CAT CT –3)
R-(5¯- CAG TGT GCA GGG TGG CAA GT-3¯
Exon-8
F-(5¯- GGA CCT GAT TTC CTT ACT CA-3¯),
R-(5¯- GAG GCA TAA CTG CAC CCT TG-3¯)

Initial denaturation at 95 oC for 2 min, 40 cycles as follows : 95 oC for1 min; 60 oC for1 min ; 72 oC for 30 sec and 5 min final extension for last cycle. The PCR products were analyzed on 2%Agarose gel electrophoresis stained with ethidium bromide.

Statistical Analysis

Frequencies and distribution of metabolic genes (gstm1 and gstt1) and P53 were used to investigate the relationship and correlation between deletion/ presence with occurrence of bladder cancer. Correlation coefficients were calculated to indicate the strength of association. The chi-square test of significance was used to reflect on the difference in the frequency of any of the two genes mutation and/ or P53 among patients and control. The ratio of the odds or the relative risk of any of the genes or gene combination was also calculated to point to the relative importance of such genes as a cause of bladder cancer.

Results:

Detection of mutations

The mutations in gstm1 and gstt1 were detected by characteristic band patterns on 2% Agarose gel electrophoresis. PCR products were 480 bp, 350 bp and 215 bp for gstt1, albumin and gstm1 genes respectively. Albumin gene was used as internal control (Figure 1).

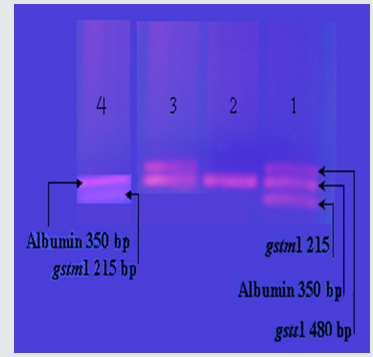


Figure 1: Patterns of PCR product for GSTs polymorphisms on 2% agarose gel at 70 voltages for one hour. Lane 1: normal genotype.Lane 2: null genotype (both genes deletion). Lane 3: gstm1 deletion. Lane 4: gstt1 deletion.

Demographical observations

Age distribution of cases were highest in age group of 51-60 years (37.14%) followed by age group of 61-70 years (25.71%) then group 41-50 years (14.29%). The percentage was 8.57% in both groups of 21-30 and 71-80 years age. 74% of the patients were males and the rest were females. The chi-square test have indicated no significant difference in incidence between males and females in both patients and control group indicating proper sampling.

The association between incidence of bladder cancer and occupation was tested using the chi-square. The test revealed high association between workers and incidence. Percent affected within each of workers, farmers, military in service and retired groups was 60%, 14.29%, 8.57%, 17.57% respectively. The low percent of affected military men do not reflects the expectation; most of the workers were ex-military men. 18 (51.43%) of the patients had Schistosomasis which is known to be connected with high incidence of bladder cancer [15]. Though the chi-square value was not significant; comparison between the observed and expected values reflected that the observed is about 25% more than the expected values within patient group, while it was less than expected within the control group.

To correlate the etiological role of aromatic amines such as.

aminobiphenyl and polycyclic aromatic hydrocarbon (which are found in cigarette and industrial chemicals), with bladder tumor genesis, the association of smoking with gstm1 and gstt1 bladder cancer risk was verified. Looking at the association of any of the mutations with smoking behavior reflects a high frequency gstm1 deletion with smoking. The observed frequency of this deletion with smoker group is higher than the expected based on the null hypothesis. The non-smoking group was characterized by the presence of both genes (gstm1 and gstt1 positive).

Polymorphism and Mutations analysis

Despite the limited number of cases , it was possible to implement basic statistical analysis (chi-square) to show the observed and expected values for bladder cancer in relation to gstm1 and gstt1 genotypes. In case of gstm1 null genotype, there was 14.29% increased possibility towards bladder cancer. In contrast with 5.7% gstt1 null genotype; there was an overall lack of association with the risk of blad der cancer (Table 1).

Table. 1: Observed and expected frequencies of mutations among patients and control.

Samples	gstm1 deletion	gstt1 deletion	Null genotypes	Normal genotypes	Total
Patients O:	5	2	10	17	35
E:	5.35	2.05	8.14	18.94	
%	14.29	5.71	28.57	48.57	
Control O:	7	3	11	29	50
E:	7.63	2.95	12.36	27.05	
%	20	8.57	31.43	82.85	
Total	31	5	21	46	85

O: Observed values. E: Expected values.Null: deletion of both genes.Normal: both genes present. X_1^2*: P>0.05.*

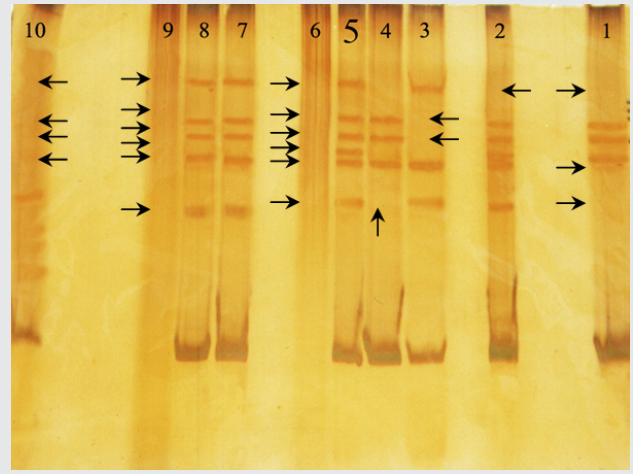
The observed frequencies are very close to the expected ones; the expected were built assuming the null hypothesis. This hypothesis is based on the assumption that the frequency of deletion and/or presence of such mutations were not different between patients and control groups. The importance of deletion/ presence of any of the metabolic genes as a risk factor was calculated and expressed as (Relative Risk) or the ratio of the odds. This will reflect the strength of developing cancer in (patients) compared to that in (control) when the gene in question is present among both!

The relative risk of both gstm1 and gstt1 deletions in this work was 1.82 which is higher than the relative risk of gstm1 or gstt1 alone. It was nearly 1.44 and 1.92 folds compared to the relative risk of gstm1 or gstt1 respectively. Other relevant studies have confirmed an association between increased risk of bladder cancer among Egyptian patients for example and deletion of gstm1, gstt1 and gstp1 [16]. However, when we calculated the relative risk, both metabolic genes were "present"; it was only 0.66, which was less than the relative risk if any or both, were absent. This might indicate that the presence of these genes do not confer a real protection and could be taken as evidence that their absence is not (a direct cause) of cancer but a predisposing factor, due to their important role in the metabolism of variety of xenobiotics including environmental carcinogens, reactive oxygen species, and chemo-therapeutic agents (1). As far as the the relationship between type of cell cancer and deletion or presence of gstm1 and gstt1 gene among patients, it was found that in Transitional Cell Carcinoma (TCC) the high percentage (28.57%) was with null genotype while with

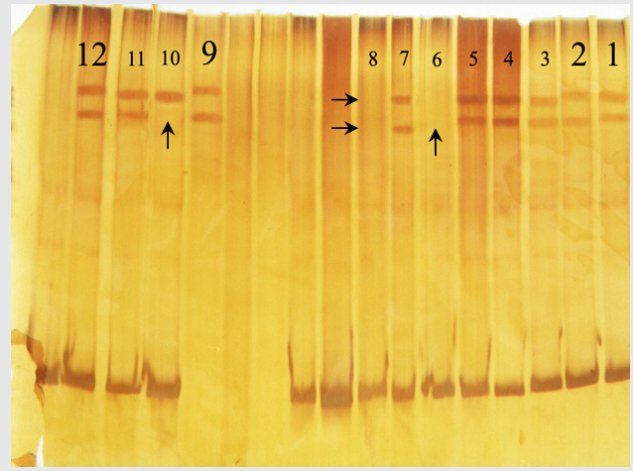
gstm1 it was 8.57% and 5.71% with gstt1. In Sequamous Cell Carcinoma (SCC) the high percentage was with gstm1 deletion (5.71%) and in null genotype (2.86%). It has however been reported that the TCC with different grade of tumor invasiveness may represent entities with different etiology and prognosis, and differences in the tumor molecular genetics [17] have reported an over expression of gstp1 and gstm1 and suggested that in the process of TCC carcinogenesis, a selection pressure occurs, resulting in a tumor with enhanced detoxification properties including that of therapeutic drugs.

One of the major tumor suppressor genes is P53. Alterations in this gene have been linked to the development of many forms of cancer, including bladder. The 35 patient samples were analyzed for point mutations in P53 gene (exon-7 and 8) by PCR- SSCP and the association with bladder cancer incidence. It turned out that 15 patients have mutations in exon-7, while 20 were normal. For control samples four cases were mutated and 46 were normal genotype. The chi-square test revealed high association between exon-7 mutations and incidence of bladder cancer; the observed value was 15, while the expected one was only 7.8 Very few number of mutations of exon-7 were found within the control group (O=4, E=11.2). For the required clarity, pattern of SSCP product (151 bp) for P53 exon-7 are shown in Figure 2 while that of exon 8 are shown in Figure 3 . Type , grade of cancer and other information for the samples are detailed in table 2. It may be worth mentioning that cases of Sequamous Cell Carcinoma (SCC) showed different banding pattern from the majority of Transitioal Cell Carcinoma (TCC) cases (sample number 28 in Fig 2 and sample 23 in Fig 3).However, the number of samples is not enough to draw a conclusion in this respect. The overall results indicate the role of P53 and occurrence of bladder cancer; the presence of these mutations confers a noticeable degree of resistance. Many reports have revealed that P53 mutations can cause not only the loss of the cell normal function, but also the gain of some new functions [18].

Figure 2: Pattern of SSCP product (151 bp) for P53 exon-7 on 12% polyacrylamide gel, on voltage 300 for 10 hour at 4°C.



Lane 1: sample no.3(TCC),Lane 2: sample no.6(TCC), ,Lane 3: sample no. 1(TCC),Lane 4: sample no.8(TCC),Lane 5: Control,Lane 6: sample no. 11(TCC),Lane 7: sample no. 1(TCC),Lane 8: sample no. 2(TCC),Lane 9: sample no. 27(TCC),Lane 10: sample no. 28(SCC)
(□): Deletion band. TCC: Trnsitional Cell Carcinoma,SCC: Sequamous Cell Carcinoma



Lane 1 and 2: Control,Lane 3: sample no. 1(TCC),Lane 4: sample no 2(TCC),Lane 5: sample no.25(TCC),Lane 6: sample no. 8(TCC),Lane 7: sample no. 24(TCC),Lane 8: sample no. 23(SCC),Lane 9: Control,Lane 10: sample no. 26(TCC),Lane 11: sample no.27(TCC),Lane 12: Control. (□): Deletion band. TCC: Trnsitional Cell Carcinoma,SCC: Sequamous Cell Carcinoma.

Table 2. Results of demographical and genetic classification of patients and control samples

No.	Sex	Age	Occupation	Smoking	Belharizasis	diagnosis	gstm1	gstt1	Null genotype	Normal genotype	Exon7	Exon 8	Exon 7+8
1	M	28	Military	Heavy	- ve	TCC GII	–	–	–	+	_	–	–
2	M	22	worker	- ve	- ve	TCC GII	–	–	+	–	_	–	–
3	M	55	worker	- ve	+ ve	TCC GIII	–	–	+	–	+	–	–
4	M	28	worker	light	+ ve	TCC GII	+	–	–	–	-	–	–
5	M	60	worker	- ve	- ve	TCC GII	–	–	–	+	-	–	–
6	M	62	Retired	- ve	+ve	TCC GII	–	–	–	+	+	–	–
7	M	55	worker	heavy	- ve	SCC GII	–	–	–	+	-	–	–
8	M	65	worker	heavy	+ve	TCC GII	–	–	+	–	–	–	+
9	M	65	worker	Heavy	+ ve	TCC GII	–	–	–	+	–	–	–
10	M	60	worker	Heavy	+ ve	SCC GII	–	–	–	+	–	–	–
11	M	74	worker	heavy	- ve	TCC GII	–	–	+	–	+	–	–
12	M	47	farmer	Light	+ ve	TCC GII	–	–	+	–	–	–	+
13	M	62	farmer	Heavy	+ ve	SCC GII	–	–	–	+	–	–	–
14	M	63	farmer	Heavy	- ve	TCC GII	–	–	–	+	–	–	–
15	M	55	woker	Heavy	- ve	TCC GII	–	–	–	+	–	–	–
16	M	60	retired	light	+ ve	SCC GII	–	–	+	–	+	–	–
17	M	68	retired	light	- ve	TCC GII	–	–	+	–	–	+	–
18	M	85	worker	- ve	+ ve	TCC GIII	–	–	–	+	+	–	–
19	M	56	Military	heavy	+ ve	TCC GIII	–	–	–	+	–	–	–
20	M	61	worker	heavy	+ ve	TCC GII	–	–	–	+	–	–	–
21	M	75		- ve	- ve	TCC GII	–	–	–	+	–	–	–
22	M	38	Military	heavy	- ve	TCC GII	–	–	–	+	+	–	–
23	M	56	worker	heavy	- ve	SCC GII	+ ve	–	–	–	+	–	+
24	M	46	retired	- ve	+ ve	TCC GII	–	–	+	–	–	–	–
25	M	50	retired	light	- ve	TCC GIII	–	–	+	–	+	–	–
26	M	50	worker	-ve	- ve	TCC GII	–	–	–	+	–	–	+
27	F	57	worker	- ve	+ ve	TCC GII	–	–	+	–	+	–	–
28	F	50	worker	- ve	- ve	SCC GIII	–	–	–	+	+	–	–
29	F	60	worker	light	+ ve	TCC GII	+	–	–	+	+	–	–
30	F	56	farmer	Light	+ ve	SCC GII	+	–	–	–	+	–	–
31	F	75	Worker	light	+ ve	TCC CIII	–	+	–	–	+	–	–
32	F	62	Worker	- ve	- ve	TCC GII	+	–	–	–	+	–	–
33	F	57	farmer	heavy	+ ve	TCC CII	+	–	–	–	–	–	–
34	F	60	worker	light	+ ve	TCC II	–	+	–	–	–	–	–
35	F	60	worker	light	+ ve	TCC GII	+	–	–	+	+	–	–

The maximum number of P53 mutations in bladder cancer among Indian population was found in exon-5 followed by exon-8 and 7. The number of mutations in exon-6 was minimal [19,20].

The long-held notion that patients with a mutated P53 gene exon-7 and 8 contract bladder cancer is illustrated by the finding in this study that there were fifteen in exon 7 (42.86%), five in exon 8 (14.38%), four combined mutations(11.42%) and eleven non mutated(normal) genotype among the patients.

The correlation coefficient between gstm1, gstt1, exon-7 and exon-8 mutations of P53 was calculated. The most important positive correlation was found between gstm1 and gstt1 which was 0.55. This was high and significant at ($P<0.01$). The other important correlation was between gstm1 and exon-7 mutation which was 0.32 ($P<0.05$). Small and non significant correlations were found between gstm1, gstt1, exon-7 and exon-8 mutations (0.281, 0.221, 0.233 respectively). It can be concluded that exon-7 of P53 deletion is more associated with gstm1, its association with gstt1 measured as correlation coefficient is very low and not significant ($r = 0.10$). The picture of mutations in gstm1, gstt1 and exon-7 being linked together is more than with exon-8 mutation as summarized in table 3.

Table 3: Distribution of gstm1, gstt1, exon-7 and exon-8 mutation among patients.

P53	gstm1 deletion	gstt1 deletion	Null genotype	Normal genotype
Exon-7 deletion	3	–	6	6
Exon-8 deletion	1	–	3	1
Exon-7 + Exon-8 deletion	1	–	2	1
Total number	5	-	11	8

Discussion:

Bladder cancer is more common in men than in women, men are approximately three times more likely to develop bladder cancer than women [4]. In this study, 74% of the patients were males and the rest were females. This might reflect a higher exposure of these patients to different carcinogens such as tobacco smoke and other environmental or occupational hazards [6]. On the other hand, androgenic hormones are known to stimulate carcinogenesis in bladder tissue, whereas estrogenic hormones block it [21]. High association (60%) with general workers in particular was noticed among the patients. Many epidemiological studies showed an increased risk of bladder cancer associated with occupational exposure. Certain carcinogens in occupational exposures cause DNA damage and may produce specific mutations [22]. Shistosomiasis is endemic in developing countries including Iraq and it is a major cause of bladder cancer. Both sequamous cell carcinoma and transitional cell carcinoma were reported [23]. There were 20 patients e suffered Shistosomiasis in this study, 16 of them have developed transitional cell carcinoma (TCC) and four sequamous cell carcinoma (Table 2). The presented analysis supports the hypothesis of Zhang [24] that certain carcinogens derived from cigarette smoking and certain occupations may induce mutations which in turn are involved in early steps of bladder carcinogenesis. However, we should note that our study is not an epidemiological constructed one, and ours are to be taken as indicators only.

The ability to characterize polymorphic genes involved in metabolism of carcinogens has opened up a new approach for human cancer risk assessment [25]. In this study, the relative risk of gstm1 deletion was 1.26 while that of gstt1 was 0.95. This might reflect the strength or degree of association and that gstm1 deletion is more associated than gstt1. In fact gstm1 polymorphism has attracted much attention owing to its possible association with an increased susceptibility to certain malignancies such as that of the bladder [6] and lung [26]. The gstm1 null genotypes show higher chance of developing bladder cancer [3]). On the other hand, some investigators [27] found no association between gstm1 deletion and bladder cancer in Indian population, while gstt1 deletion increased the risk. In this work, gstt1 genotype did not present any significant association with bladder cancer.

The P53 mutation spectra were found to be variable in different populations. This indicates that risk factors are not the same from one population to another [28]. This could also affect the importance of gstm1 and gstt1 gene products in detoxifying carcinogens [29.30]. Low or deficient activities of conjugation enzymes of foreign compound metabolism may influence types of acquired mutations in P53 exons 4-9 in bladder cancers [30,31]. Several genetic alterations, including the inactivation of tumor suppressor genes or the activation of oncogenes are considered to be the main reasons of carcinogenesis [32,33].

The results substantiated that not only the genes (GSTs and P53), neither only the environmental factors

are associated with bladder cancer development, ethnic correlation may be another factor need to be explored. The relation of gstm1 and gstt1 genes with the incidence of bladder cancer was not studied in this population before. It is thus difficult to conclude what is the exact environmental change that had the direct impact particularly with the fact that the region has been a victim of vicious wars against living beings and the environment.

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Conflicts of interest:

The authors declare that no conflict of interest exists.

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التباين الوراثي لجيني الـ P53 &GSTs بين مرضى سرطان المثانة في العراق

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الخلاصة:

تضمنت الدراسة تحديد دور الجينات الايضية (gstm1, gstt1) وجين P53 لـ (exon7-8) وبعض العوامل المسببة للإصابة بسرطان المثانة بين المرضى في العراق كعوامل خطورة.

تم جمع عينات دم من 35 مريض مشخص بالإصابة بسرطان المثانة من مراجعي مستشفى الطب النووي في بغداد ومنهم 24 ذكور و9 إناث. كما شملت عينات السيطرة 50 حالة من الأصحاء ظاهرياً (إناثاً وذكور).

تضمنت الدراسة تحليل العوامل التالية العمر، الإصابة بالبلهارزيا , نوع العمل والتدخين، لإيجاد العلاقة بين العوامل البيئية والإصابة بسرطان المثانة تم استخلاص الحامض النووي (DNA) باستخدام طريقة الهضم بالـ (Proteinase K/SDS) ولدراسة التحليل الجيني لهذه العينات استخدمت طريقة التضاعف السلسلي المزدوج (multiplex PCR) للحامض النووي للكشف عن وجود جينات gstm1 و gstt1 بالإضافة إلى جين الألبومين كدليل سيطرة (موجبة) وعلى الرغم من أن هذا الاختبار لا يميز بين الكميات المتماثلة والمتشابهة إلا أنه يميز بينها عن طريق الحذف الكلي للجين.

بالإضافة إلى تقنية التضاعف التسلسلي تم استخدام تقنية (Single Strand Conformation Polymorphism SSCP) الترحيل على هلام البولي اكريل امايد للكشف عن الطفرة النقطية لجين P53 ولكل من الـ exon7, 8.

كشفت النتائج على أن النسبة المئوية لمرض سرطان المثانة كانت ٪37.14، للفئة العمرية 65-51 سنة ومعظمهم من الذكور (٪74.29)، ٪60 من المرضى كانوا يعملون أعمال مختلفة (٪17.14) متقاعدون ,(٪14.29) فلاحين و٪8.57 عسكريين.

أثبتت الدراسة وجود علاقة بين الإصابة بالبلهارزيا وسرطان المثانة وبنسبة (٪51.43) من المرضى المصابين سابقاً بينما (٪48.57) لم يكونوا مصابين.

التحليل الوراثي أثبت أن حدوث الطفرة (حذف ألجين كلياً) لجين gstm1 كان مرتبط إحصائياً بسرطان المثانة وبنسبة ٪14.29 هذا الارتباط كان أقوى في حالة حدوث الطفرة الوراثية لجين (gstt1, gstm1) وبنسبة ٪28.5. كما أظهرت النتائج وجود ارتباط قوي بين حدوث الطفرة في gstm1 وسرطان المثانة عند المدخنين والمصابين بالبلهارزيا وعدد الطفرات في جين P53 كان أكثر في المرضى الذين لديهم طفرة مزدوجة لجيني (gstm1, gstt1) أو لجين gstm1 فقط.

بينت النتائج وجود ارتباط عالي بين حدوث الطفرة في جين P53 و(exon7) و الإصابة بسرطان المثانة وبنسبة ٪42.86 بينما الارتباط بين الطفرة لـ جين P53 لـ (exon8) وسرطان المثانة كان قليل وبنسبة ٪14.28.

كذلك أظهرت النتائج أن حدوث الطفرة في جين gstm1 و exon7 مرتبطين معاً أكثر من حدوثها في exon8.