Ames test For the Detection of Mutagens in the Urine of Egyptian Cancer Patients

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ABSTRACT

Introduction: *The analysis of urine for mutagenic substances could provide means both for verifying suspected, and for detecting otherwise unrecognized substances that may be capable of damaging the human genome.* ***Aim of Work:*** *This work aims to study the possible mutagens present in the unconcentrated human urine of a variety of cancer patients; namely bladder, breast and liver, using Ames test for mutagenicity of Salmonella species.* ***Materials and methods****: Urine samples from 121 cancer patients were tested; 64 bladder cancer, 32 hepatocellular carcinoma (HCC), and 25 breast cancer patients. Sixty five non cancer, apparently healthy subjects were tested as controls. Four strains of histidine requiring* ***Salmonella Typhimurium*** *LT2 bacteria were used; namely TA 97, TA 98, TA 100 and TA 102. Each tester strain carries a different type of mutation (defect) in the histidine operon.* ***Results:*** *Mutagenicity values were obtained in urine of bladder cancer patients amounting to 27% (p= 0.007), 34.4% (p<0.001), 25% (p=0.001), and 21.9% (p=0.01) for tester TA 97, TA 98, TA 100 and TA 102 strains; respectively. Urine of liver cancer patients gave mutagenicity values of 3.1%, 0%, 56% (p= 0.001), 19% ; while in the urine of breast cancer patients showed mutagenicity values of 16%, 8%, 32% (p= 0.01), and 16% for tester TA 97, TA 98, TA 100 & TA 102 strains; respectively. Normal subjects showed mutagenicity of 18%, 9%, 16.4%, and 3% for the same tester strains; respectively. Our results showed that the urinary Ames test seems to be related to smoking and urinary bacterial infection in bladder cancer patients. It is also related to the inefficient detoxification in the liver cancer patients; as well as the urinary bacterial infection in non-smoker females with breast cancer.* ***Conclusions:*** *Urinary Ames test, besides being efficient in detecting urine mutagens, it has the potential of being an easy and quick survey technique for detecting mutagens in high-risk subjects.*

Key words: *Salmonella Typhimurium, Ames test, urine, Cancer patients.*

INTRODUCTION

Bladder, liver and breast carcinomas are the most common types of cancer among the Egyptian population. Carcinoma of the bladder is the first and most common form of malignancy seen in males treated at the National Cancer Institute, Cairo University; representing 18% of all male malignancies, 1680 cases were admitted during the period from January 2002 till December 2003. However, 525 females were seen with bladder cancer during the same period, representing 5.7% and ranked the fifth among all female cancers. Regarding liver cancer, there were 1,055 (11.3%) males and 339 (3.7%) females, and their median age was 57 years. Liver cancer ranked the second among males and the seventh among females. On the other hand, there were 82 males (0.9% of all cancer types in men) and 3,437 females (37.5% of all cancer types in women) with breast cancer, where it ranked number one among females[1].

Relatively, little is known about the etiology of bladder cancer; however, most evidences favor a strong role for chemical carcinogens[2]. Etiological factors reported include occupational exposure to chemicals, cigarette smoking, dietary factors (including high nitrite ingestion, possibly resulting in nitrosamine formation), and metabolic products from secondary bacterial and parasitic infections, bladder calculi, a direct physical effect of the *S. haematobium* (bilharziasis) ova, or additional unrecognized factors[2-8].

In view of the fact that detoxifying enzymes, which are responsible for detoxification of tobacco-borne carcinogen detoxification and cellular protection, were found to be low in hepatocellular carcinoma patients[9]. Also, malignancy, immunosuppressive therapy, female gender, and hospitalization are risk factors for bacterial

urinary tract infection[10], which might allow for the formation of carcinogens in female patients with breast cancer by the same mechanism as bladder cancer patients do.

Ames test is a biological assay used to test for mutagenic properties of a chemical compounds. This assay is carried out using strains of bacteria generally *Salmonella Typhimurium* that already have a single mutation. The set of histidine-requiring bacteria is used for mutagenicity testing. Each tester strain contains a different type of mutation in the histidine operon. In addition to the histidine mutations that greatly increase their ability to detect mutagens. One mutation (*rfa*) causes partial loss of lipopolysaccharide barrier that coats the surface of the bacteria and increases permeability to large molecules such as benzo(α) pyrene that do not penetrate the normal cell wall[11]. The other mutation (*uvrB*) is a deletion of a gene coding for the DNA excision repair system, resulting in generally increase sensitivity in detecting many mutagens. T102 does not contain the *vurB* mutation because it was constructed primarily for detecting mutagens that require an intact excision repair system. The standard tester strains TA97, TA98, TA100, and TA102 contain the R-factor plasmid pk M101. TA102 also contains the multicopy plasmid, pAQ1, which carries hisG428 mutation and a tetracycline resistance gene. These R-factor strains are reverted by a number of mutagens that are weakly detected or even not detected at all with the non R-factor parent strains[11,12].

In brief, TA100 strain was developed to detect hard methylating agents such as dimethylnitrosamine, TA102 could detect cross-linking agents such as mitomycin C which require the R-factor and an intact excision repair system[13], TA97 could detect certain frameshift mutagens[14]. On the other hand, TA100, and TA98 are useful for studying the metabolism and mutagenicity of nitro carcinogens such as nitrofurazone and furyfluramide which are activated directly to mutagens by bacterial nitroreducatses[15].

A rather old study, Ames and McCann[16] showed that the correlation between carcinogenicity and mutagenicity is about 83%. Some of the carcinogens that were reported to be negative can now be detected using the new tester strains. For example, the addition of cofactors to the S9 mix allows the detection of certain azo dyes.

Aim of work: Using Ames test, we attempted to identify possible formation of mutagenic substances in the urine of bladder cancer patients. Also we were intrigued to test patients with HCC due to the low carcinogen detoxification, and a group of patients with breast cancer as cancer control group, and correlating Ames positivity with the clinical findings.

MATERIALS & METHODS

Urine specimens:

Urine samples were taken randomly from 121 cancer patients prior to treatment, presented to the out clinic of National Cancer Institute, Cairo University, starting from January 2001 and June 2002. This comprised 64 bladder cancer patients, 32 hepatocellular carcinoma patients and 25 breast cancer patients. Sixty five apparently healthy subjects (relatives of patients and non-health care workers at the National Cancer Institute, Cairo University) were also tested as controls. All urine samples were subjected to testing by Comber 9 strips for biochemical analysis (Boehringer Mannheim, USA). It measures pH and specific gravity of urine as well as the presence of sugar, protein, Ketone, nitrite, Bilirubin and urobiliogen in urine. Urine was also subjected to routine microscopic examination and bacteriological cultures. Urine samples were then filter-sterilized using individual 0.45 micron filters (Millipore Corp., USA), aliquots were deep frozen at -80°C till used in the Ames test.

Urine bacterial culture:

Fifty micro liters of the freshly collected urine were inoculated in nutrient agar and blood agar media. The bacterial strains were identified using standard API kits.

Bacterial strains:

Four strains of histidine requiring ***Salmonella Typhimurium*** LT2 bacteria were used, namely TA 97, TA 98, TA 100 and TA 102 for the study. They were obtained as a gift from Dr. Bruce Ames laboratories, University of California, USA. Periodic testing revealed appropriate sensitivity to ampicillin and crystal violet. Each tester strain carries a different type of mutation (defect) in the histidine operon. They were lyophilized and when dissolved, they were aliquoted and stored at -80°C till used[17].

Chemicals:

The NADP, NADPH, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, and Beta-glucuronidase were purchased from Sigma

Chemical Company, USA. L-histidine was purchased from BDH Biochemicals, England.

Media:

Sterile media and bacteriologic plates were prepared, including phosphate buffered saline, pH 7.4 (PBS); nutrient broth (8 g/L oxoid agar with 8 g/L NaCl); minimal glucose plates (1.5% Oxoid Agar, 20ml/L 50 times concentrated Vogel-Bonner salts, 2.0% glucose); and top agar (0.6% oxoid agar, 0.5% NaCl, 96 mg/L of L-histidine, and 120.4 mg/L biotin) with 20 ml of medium per sterile plastic Petri dish (9 cm diameter).

Preparation of rat liver homogenates:

Sprague-Dawley male rats weighing approximately 200 grams were used. Rats were given sodium phenobarbital 0.1% in drinking water for 5 days in order to induce the liver enzymes of the rats. Food, not water, was removed 12 hours before being sacrificed at the sixth day. Freshly excised liver was placed in pre-weighed beakers containing approximately 1ml of chilled 0.15 M KC1 per gram of wet liver. The liver was then washed several times in fresh, chilled 0.15 M KC1 in order to remove hemoglobin which can inhibit the activity of P450 enzymes and to ensure sterility of the preparation. Washed liver was transferred to a beaker containing 0.15 M KCl in a ratio of 3ml/g of wet liver and minced with scissors and homogenized using a polytron homogenizer. The homogenate was centrifuged for 10 min. at 8,700 rpm; the supernatant (the S9 fraction) was decanted and saved. The freshly prepared S9 fraction was distributed in 1-2 ml portions in small plastic Nunc tubes, frozen quickly in a bed of crushed ice, and stored immediately at -80ºC. For purposes of checking the sterility of the preparation, 0.1 ml of the S9 fraction was plated on minimal agar containing biotin and histidine[17].

Mutagenesis assay:

Thirty milliliter of minimal glucose agar medium was poured in each Petri dish and left to solidify. For the plate incorporation test, each plate combines the urine, the bacterial strain, and S9 mix in soft agar which is poured onto a minimal agar plate. This was done by adding histidine and biotin to the top agar as mentioned above. Two milliliter of top agar was distributed in 10 ml tissue culture tubes held at 45ºC water bath. Followed by the addition of 0.1 ml of a fresh overnight culture of the tester strain. Also, 0.1ml of urine and 0.5 ml of S9 mix were added. Positive and negative controls were also included in each assay. After incubation at 37ºC for 48 hours, revertant colonies were counted.

Statistical analysis:

SPSS package (version 13) was used. Chi-square and when necessary, Fisher-exact test were used to test proportion independence. For more than 2X2 tables with significant results, pair wise comparisons were done with adjustment of α level for the number of comparisons done. Kruskal Wallis test was used for comparing quantitative data for >2 groups. The level of probability of ≤ 0.05 is considered significant.

RESULTS

Table (1): Clinical features and complete urine analysis of the study groups

Parameter	Diagnosis			NCC (n=65)	P value
	Bladder ca. (n=64)	HCC (n=32)	Breast ca. (n=25)		
Urine analysis:					
Sp Gravity	1.010	1.025	1.020	1.025	<0.001
Median (range)	(1.00-1.10)a	(1.01-1.03)b	(1.01-1.03)b	(1.01-1.03)b	
Nitrite	13 (20%)	0 (0%)	0 (0%)	0 (0%)	<0.001
Protein +ve	50 (78%)	9 (28%)	8(32%)	19 (29%)	<0.001
Glucose +ve	0 (0%)	11 (34%)	4 (16%)	8 (12%)	<0.001
pH	6	5	5	5	<0.001
Median (range)	(5-9)a	(5-5)b	(5-6)b	(5-9)b	
Ketone +ve	0 (0%)	2 (6%)	2 (8%)	0 (0%)	0.02
Urobilinogen +ve	18 (28%)	24(75%)	10 (40%)	8 (12%)	<0.001
Bilirubin +ve	12 (19%)	6 (19%)	0 (0%)	2 (3%)	0.003
WBCs +ve	59 (92%)	10 (32%)	6 (24%)	5 (8%)	<0.001
RBc's +ve	59 (92%)	10 (32%)	4 (16%)	5 (8%)	<0.001
Bacteria +ve	37 (57.8%)	25 (78%)	18 (72%)	12 (18.5%)	<0.001

p≤ 0.05 is significant, groups sharing the same letters are not significantly different.
NCC = Non Cancer Controls. HCC = Hepatocellular Carcinoma

All the clinical data and the complete urine analysis of the 121 cancer patients as well as the 67control apparently healthy subjects are presented in table (1). Breast cancer patients were all females but male to female ratio was 4.1:1, 7:1, 3.8:1 in bladder cancer group, HCC group, and NCC group respectively. Their ages were 55±9, 58±8.5, 50±10, 36± 10 years for bladder cancer group, HCC group, breast cancer group, and NCC group respectively. Forty one percent, 59%, 23% of bladder cancer patients, HCC patients, and NCC group were smokers whereas none of the breast cancer patients were smokers. Regarding the tumor grade among patients with HCC, breast, and bladder cancer 12.5%, 22%, and 16% were grade 1, 47%, 44%, and 36% were grade 2 and 40.5%, 34%, and 48% were grade 3 with no significant difference. Stages 2 and 3 of the tumor constituted almost two thirds of cases in the different cancer groups.

Regarding urine analysis using Comber 9 strips and the microscopic examination, 20% of bladder cancer patients showed nitrite in their urine, whereas it was absent in the urine of the other groups investigated. Eleven patients (34%) with HCC had glucose in urine in comparison to 0%, 16%, and 12% in bladder cancer, breast cancer and normal controls; respectively. The acidic pH of urine was common among all groups except the bladder cancer patients who showed alkaline pH in 20% of the cases. Eighteen patients (75%) of HCC patients had urobilinogen in their urine in comparison to 28%, 40%, and 12% in bladder cancer, breast cancer and normal controls respectively. Bladder cancer patients had more bilirubin, RBCs, and WBCs than in all other groups. Cancer patients showed different bacterial infection in their urine with a prevalence greater than in the control subjects.

Table 2: Patients with reversion of strains used in the Ames test in the different study groups

Group (No.)	**Patients with revertant strains**				**Total revertants No. (%)**
	TA 97 No. (%)	**TA 98 No. (%)**	**TA 100 No. (%)**	**TA 102 No. (%)**	
Bladder cancer (n=64)	17 (27%)	22 (34.4%)	16 (25%)	14 (21.9%)	37 (58%)
HCC (n=32)	1 (3.1%)	0 (0%)	18 (56%)	6 (19%)	25 (78%)
Breast cancer (n=25)	4 (16%)	2 (8%)	8 (32%)	4 (16%)	18 (72%)
Controls (n=65)	6 (9%)	0 (0%)	11 (17%)	2 (3%)	12 (18.5%)
P value	0.007	<0.001	0.001	0.01	<0.001

p≤ 0.05 is significant

The frequency of histidine revertants per plate for individual urine and control samples using strains TA97, TA98, TA100, and TA102 are listed in table (2), which shows that total revertants and revretant strains were compared among the 4 studied groups and when significant, control group was tested versus the 3 cancer groups for differences. On pair wise comparison, it was found that bladder cancer patients versus controls were significantly different regarding total revertants and revretant strains with the exception of strain TA100. But when comparing bladder patients versus HCC and breast cancer groups, all were significantly different but not with strains TA102 and TA98; respectively.

There was a significant increase among plates treated with urine from bladder cancer patients (3 folds of urine from normal subjects) and from HCC patients (2 folds of urine from normal subjects) as well as from breast cancer patients in comparison with plates treated with urine from normal subjects. In comparison to normal subjects: bladder cancer patients showed a significant increase in revertants for all the four strains, HCC patients showed a significant increase in revertants for strain TA100 only, whereas breast cancer patients showed a statistically significant higher number of revertants of TA 100 strains.

Regarding smoking habit, smokers represented 26/37 (70%), 18/25 (72%), 10/12 (83%) of those with positive Ames test in the bladder cancer, HCC, and controls, in comparison to 10/27 (37%), 2/7 (28%), 8/55 (15%) in those negative for Ames test; in the same groups respectively. (Tables 4- 7).

Table (3): Histology, grade, and lymph node involvement in relation to positivity of Ames test in bladder cancer patients

Parameter	Ames test		*P* value
	Positive (n= 37)	**Negative (n=27)**	
Histological type:			0. 13
SCC	13 (35.1%)	13 (48.1%)	
TCC	14 (37.8%)	12 (44.4%)	
Others	10 (27%)	2 (7.4%)	
L. N. involvement:			<0.001
Yes	35 (95%)	15 (55.6%)	
No	2 (5%)	12 (44.4%)	
Tumor grade:			<0.001
1+2	15 (40.5%)	23 (85%)	
3	22 (59.5%)	4 (15%)	

p≤ 0.05 is significant *LN = Lymph node*
TCC= Transitional cell carcinoma *SCC = Squamous cell carcinoma*

The histological type of tumor was available only in cancer bladder patients and its relation to Ames test is shown in table (3). The squamous cell carcinoma and the transitional cell carcinoma cell types had no impact on the positivity of Ames test. On the other hand, the eight cases with mixed cell type were all positive for Ames test. On the other hand, 35 (95%) of bladder cancer with lymph node metastasis were positive for Ames test in comparison to 55.6% only in those with no reactivity for Ames test. Also, most of those with tumor grade 3 were positive for Ames test.

Table (4): Comparison of the clinical and laboratory results between Ames positive and Ames negative cases in the bladder cancer group

Parameter	Bladder ca. Ames +ve (n=37) N (%)	Bladder ca. Ames -ve (n=27) N (%)	*P* value
Sex:			
M/F	30/7	22/5	0.968
Age (Mean ± SD)	54 ± 8.4	56 ± 9.9	0.470
Smoking	26 (70%)	10 (37%)	<0.001
Urine analysis:			
Sp Gravity	1.0125	1.00	0.113
Median (range)	(1.00-1.10)	(1.00-1.10)	
Nitrite	12(32.4%)	1 (3.7%)	0.005
Protein +ve	29 (78%)	21 (77.8%)	0.954
pH 5	5	6	0.15
Median (range)	(5-9)	(5-9)	
Urobilinogen + ve	8 (21.6%)	10(37%)	0.176
Bilirubin +ve	4 (10.8%)	8 (29.6%)	0.057
WBCs +ve	36 (97.3%)	23(85.2%)	0.153
RBc's +ve	32 (86.5%)	27 (100%)	0.065
Bacteria +ve	37 (100%)	17 (63%)	<0.001

p≤ 0.05 is significant

Table (5): Comparison of the clinical and laboratory results between Ames positive and Ames negative cases in the hepatocellular carcinoma group

Parameter	HCC Ames +ve (n=25) N (%)	HCC Ames -ve (n=7) N (%)	*P* value
Sex:			
M/F	23/2	5/2	0.201
Age (Mean ± SD)	57 ± 7.6	61 ± 11.5	0.47
Smoking	18 (72%)	2 (28%)	0.01
Urine analysis:			
Sp Gravity	1.03	1.03	0.562
Median (range)	(1.02-1.02)	(1.02-1.03)	
Protein +ve	8 (32%)	1 (14.3%)	0.357
Glucose +ve	9 (36%)	2 (28.6%)	1.0
pH 5	5	5	1.00
Median (range)	(5-5)	(5-5)	
Ketone + ve	2 (8%)	0 (0%)	1.0
Urobilinogen + ve	20(80%)	4 (57.1%)	0.217
Bilirubin +ve	6 (24%)	0 (0%)	0.296
WBCs +ve	7 (29%)	3 (42.9%)	0.652
RBc's +ve	9 (36%)	1 (14.3%)	0.387
Bacteria +ve	12 (48%)	1 (14.3%)	0.195

$p \leq 0.05$ is significant

Table (6): Comparison of the clinical and laboratory results between Ames positive and Ames negative cases in the breast cancer group

Parameter	Breast ca. Ames +ve (n=18) N (%)	Breast ca. Ames -ve (n=7) N (%)	*P* value
Sex:			
M/F	0/18	0/7	
Age (Mean ± SD)	49 ± 11	52 ± 4.1	0.57
Urine analysis:			
Sp Gravity	1.02	1.025	0.974
Median (range)	(1.01-1.03)	(1.01-1.03)	
Protein +ve	6(33%)	2 (28.6%)	1.0
Glucose +ve	3 (17%)	1 (14.3%)	1.0
pH 5	5	5	0.836
Median (range)	(5-6)	(5-6)	
Ketone + ve	0 (0%)	2 (28.6%)	0.07
Urobilinogen + ve	10 (56%)	0 (0%)	0.02
Bilirubin +ve	0 (0%)	0 (0%)	
WBCs +ve	6 (33%)	0 (0%)	0.137
RBc's +ve	4 (22%)	0 (0%)	0.294
Bacteria +ve	15 (83%)	0 (0%)	<0.001

$p \leq 0.05$ is significant

Table (7): Comparison of the clinical and laboratory results between Ames positive and Ames negative cases in the non cancer controls

Parameter	**Controls Ames +ve (n=12) N (%)**	**Controls Ames -ve (n=53) N (%)**	***P* value**
Sex:			
M/F	11/1	41/12	0.237
Age (Mean ± SD)	36 ± 11	41 ± 7.6	0.93
Smoking	10 (83%)	8 (15%)	<0.001
Urine analysis:			
Sp Gravity	1.0275	1.025	0.349
Median (range)	(1.02-1.03)	(1.01-1.03)	
Protein +ve	4 (33%)	15 (28.3%)	0.673
Glucose +ve	2 (17%)	5 (9.4%)	0.577
pH 5	5	5	1.00
Median (range)	(5-5)	(5-5)	
Urobilinogen + ve	2 (17%)	5 (9.4%)	0.577
Bilirubin +ve	0 (0%)	2 (4%)	1.0
WBCs +ve	2 (17%)	3 (5.6%)	0.216
RBc's +ve	2 (17%)	3 (5.6%)	0.216
Bacteria +ve	7 (58%)	5 (9.4%)	<0.001

p≤ 0.05 is significant

A comparison of the clinical and laboratory results between Ames positive and Ames negative cases in the different study groups is shown in tables (4-7). Bladder cancer patients positive for Ames test showed more urine nitrite, bilirubin, WBCs, RBCs and bacteria in culture. Whereas, breast cancer patients and controls positive for Ames test had more frequently positive bacterial culture in the urine.

Table (8): Bacterial isolates of the study groups

Bacterial culture isolates	**Bladder cancer (n=64)**		**HCC (n=32)**		**Breast cancer (n=25)**		**Controls (n=67)**	
	M (n=52)	**F (n=12)**	**M (n=28)**	**F (n=4)**	**M (n=0)**	**F (n=25)**	**M (n=52)**	**F (n=13)**
Culture +ve	30 (58%)	7 (58%)	23 (82%)	2 (50%)	0 (0%)	18 (72%)	7 (13%)	5 (36%)
Citrobacter freundii	1	0	0	0	0	3	0	1
Citrobacter koseri	1	1	1	0	0	2	0	0
E.coli	8	3	7	0	0	7	2	1
Hafniaalvei	1	0	0	0	0	2	0	0
Morganella morganii	2	1	1	0	0	3	0	0
Neisseria gonorrhoeae	3	2	2	0	0	2	0	0
Proteus mirabilis	3	2	4	0	0	1	0	0
Providencia rettgeri	1	0	0	0	0	0	0	0
Pseudomonas aerugenose	6	0	2	0	0	2	1	0
Salmonella spp	0	0	2	0	0	0	1	1
Seratia marcescens	1	2	1	0	0	3	1	0
Staphylococcus aureus	10	5	7	1	0	0	0	1
Staphylococcus saprophyticus	2	1	1	0	0	0	0	1
Enterococcus feacalis	5	1	2	1	0	1	1	1
Total no of isolates	44	18	30	2	0	26	6	6

Table 8 shows the frequency of bacteria in the different study groups. Staphylococcus species, Enterococcus, and E-coli were the most common types of bacteria isolated.

Table (9): Results of multivariate logistic regression analysis for no of revertants as outcome and disease type, smoking, and urine bacterial culture results as independent variables

	B	**S.E.**	**p value**	**Odds ratio**	**95.0% C.I. for OR**	
					Lower	**Upper**
groups (CONTROL) Ref			<0.001			
groups (baldder)	.990	.772	.200	2.692	0.592	12.234
groups(HCC)	4.107	.919	<0.001	60.766	10.039	367.824
groups(Breast)	4.235	.901	<0.001	69.083	11.826	403.547
smoking	3.957	.758	<0.001	52.279	11.829	231.048
bacteria	3.369	.743	<0.001	29.053	6.775	124.578

To adjust for differences in distribution of age and sex found on univariant analysis between the 4 studied groups, multivariate logistic regression analysis was done where total number of revertants in the Ames test was the dependent (outcome) variable and age, sex, grouping variable (disease, no disease), smoking and bacterial culture in urine were the independent variables. Results, as shown in table (9), verified that revertants in Ames test was highly dependent on smoking status OR= 52 (11.8-231), bacterial culture in urine OR=29 (6.7-125) and types of cancer, where HCC group was significantly affecting the total number of revertants compared to controls (OR=60 (10-367.8) and also breast cancer compared to controls (OR= 69(11.8-403) and $p<0.001$ but not for bladder cancer. Age and sex were found to have no effect on the total number of revertants (p=0.1, 0.88 respectively).

DISCUSSION

Ames and associates previously described a very sensitive and simple bacterial test for detecting chemical mutagens. The compounds are tested on Petri plates with several especially constructed mutants of *Salmonella Typhimurium* selected for sensitivity in being reverted from a histidine requirement back to prototrophy by a wide variety of mutagens. Unlike chemical analyses, the analysis of mutagenic substances in body fluids by Ames test can detect effects of many substances, including unanticipated compounds and metabolites and compounds formed by interactions between individual substances in complex mixtures[17].

Previously published work were also able to detect mutagenic activity in unconcentrated human urine, using Ames test, from individuals exposed to compounds including nitrofurantoin[18], metronidazole[19,20] and several agents used for cancer chemotherapy[21]. In addition, human urine from smokers[22] polycyclic aromatic hydrocarbon[23], patients exposed to metronidazole[24] or workers exposed to epichlorohydrin[25] had mutagenic activity after concentration by solvent or resin extraction or by lyophilization. In the studies sited mutagenic activity was not detected for unexposed controls. Regarding the preferable use of urine filtrate over urine sediment, it was found that the mutagenic effect of urine should be tested only in urinary filtrate, whereas there were no detectable mutagenic substances in urinary sediment[26].

On the other hand, a study done by Rosin et al.[27] indicated that chromosome-damaging (clastrogenic) compounds can be produced during bacterial infections in the urinary bladder, and supports a direct involvement of urinary tract infection in the development of bladder cancer. This is in agreement with our results; bladder and breast cancer patients; as well as controls positive for Ames test had more frequently positive bacterial infection in urine. However, HCC patients positive for urine bacterial culture did not differ significantly regarding Ames test positivity. This might be explained by the fact that chronic liver disease accounts for this positivity rather than the bacterial infection in urine. In a study by Sivestri and associates[28] who found that polymorphic variants of enzymes involved in the metabolic activation/detoxification of carcinogens may contribute to the progression of liver disease and HCC risk. Also detoxifying enzymes capable of tobacco-borne carcinogen detoxification and cellular protection were found to be low in hepatocellular carcinoma patients[29].

Cigarette smokers have been reported to void urine which is more mutagenic, as measured in the Ames test, than urine voided by non-smokers[30]. This is in agreement with our findings, where a statistically significant percent of smokers were positive for Ames test. Several data showed that cigarette smokers' urine is highly mutagenic, whereas non-smokers resulted in a very weak mutagenic activity of urine[30,31]. Thus Ames test could be used to determine the mutagenicity level of smokers, also it was suggested that it is applicable for urines of ex-smokers and passive smokers[32]. The fact that breast cancer patients were all female non-smokers could indicate that their urine mutagenicity has no relation to smoking, however, passive smoking could contribute to the high mutagenicity in their urines or urinary bacterial infection. This theory is yet to be verified since this data about passive smoking is missing. On the other hand, smoking is one of the etiological factors of bladder and liver cancer[33,34]. In agreement, we noticed a statistically significant percent of smokers among bladder and liver cancer patients in comparison to the controls.

Cystitis-induced bladder cancer (from all causes) is usually associated with severe long term bacterial infections. The mechanisms of

carcinogenesis are not understood but may involve formation of N-nitroso compounds in the bladder[35,36]. This is in agreement with our findings.

El-Aaser et al.[37] and El-Aaser et al.[8] reported that bacterial flora in urine posses two biological activities; first, reduction of nitrate to nitrite which in turn in presence of secondary amines and acidic pH of the urine(most of our patients have a urine pH = 5) can form a potent carcinogen, namely, nitrosamines. The second activity of bacteria is the hydrolysis of conjugated carcinogen converted it to unmasked active carcinogen through its active beta-glucoronidase. This is in addition to secreting exotoxin in the urine which might be mutagenic or carcinogenic. Adding to this, chemical and mechanical irritation of the urothelium associated with hyperplasia usually induced, which favor the mutagenic action of the formed carcinogen.

The injury of the urothelium predisposes to secondary bacterial infection, which in turn contributes to a rise of beta-glucuronidase of tissue origin in urine. This can lead to various hyperplastic and metaplastic lesions. Tissue Beta-glucuronidase, which has optimal activity at pH 5, can hydrolyze many conjugated carcinogens into non-conjugated active ones. In addition, the mucosal barrier effectiveness of the injured urothelium to reabsorb carcinogens in urine is usually reduced [37,38].

In conclusion: Mutagenic substances in the urine of cancer patients could be efficiently detected. Ames positivity in the examined urine might be attributed to formation of mutagens due to bacterial infection and smoking in case of the bladder cancer patients and due to impaired detoxification mechanism due to liver disease in case of the liver cancer group, whereas it might be attributed to bacterial infection in case of female breast cancer patients. Furthermore, studies are needed to determine the origin of chemicals and genotoxic characteristics of urinary mutagens.

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اختبار Ames للكشف عن المطفرات فى البول فى مرضى السرطان من المصريين

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المقدمة: قد يقدم تحليل البول للكشف عن المواد المطفرة وسيلة إما للتيقن من المواد المشكوك فيه أو للكشف عن المواد الغير معرفة بخلاف ذلك والتى لها القدرة على احداث الضرر للجينوم البشرى.
هدف الدراسة: تهدف هذه الدراسة الى الكشف عن المطفرات المحتمل وجودها فى البول الادمى غير المركز لمرضى سرطانات مختلفة: منها سرطان المثانة، الثدى، والكبد باستخدام اختبار ال Ames للمطفرات للسلالة Salmonella
طرق البحث: قمنا بدراسة على عينات البول ل ١٢١ مريض سرطان: منهم ٦٤ مريض سرطان مثانة، ٣٢ مريض سرطان كبد، و٢٥ مريضة بسرطان الثدى. تم كذلك الدراسة على عدد ٦٥ فرد اصحاء ظاهريا غير مرضى بالسرطان كمجموعة قياسية. تم استخدام ٤ سلالات من بكتريا Salmonella Typhimurium وهى TA97, TA98, TA100, TA102.
وكل سلالة تحمل طفرة مختلفة (نقص) فى متجاورة جين ال histidine.
النتائج: وجدت نسبة الطفرات فى البول لمرضى سرطان المثانة فى ٢٧% (ت= ٠،٠٠٠٧) ٣٤،٣% (ت<٠،٠٠٠١) و ٢٥% (ت= ٠،٠٠٠١) و ٢١،٩% (ت= ٠،٠٠١) للسلالات تحت الدراسة TA98,TA97, TA102,TA100 ,على التوالى. وأعطى البول فى مرضى سرطان الكبد نسب تطفر ٣،١%و ٠% و ٥٦% (ت= ٠،٠٠٠١) و ١٩% ، بينما فى البول لمرضى سرطان الثدى فقد أعطى النسب ١٦% و ٨%و ٣٢% (ت= ٠،٠٠١) و ١٦% للسلالات تحت الدراسة TA102,TA100 , TA98,TA97,على التوالى. كما أعطى البول فى المجموعة القياسية القيم ١٨% و ٩% و ١٦،٤% و ٣% لنفس السلالات على التوالى. وقد أظهرت هذه الدراسة أن اختبار ال Amesقد يكون له علاقة بالتدخين والعدوى البكتيرية فى البول فى مرضى سرطان المثانة. كما أن له علاقة ايضا بالتخلص غير الكفؤ من السموم فى مرضى سرطان الكبد، كما أن له علاقة بالعدوى البكتيرية فى مريضات سرطان الثدى من غير المدخنات.
المستخلص: كان لاختبار ال Ames بجانب كفائته فى الكشف عن المواد المطفرة فى البول فان له القدرة على أن يكون اختبار ماسح بسيط وسريع للكشف عن المطفرات فى الأفراد الأكثر عرضة.