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A Study on the Aetiological Factors of Bilharzial Bladder Cancer in Egypt—1 Nitrosamines and their Precursors in Urine

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Abstract—*Endogenous nitrosamines and nitrosamines formed by deliberate chemical nitrosation of urine have been found to be much higher in urine of bilharzial infested and bladder cancer patients when compared with that of normal or cancer patients other than bladder cancer. Thiocyanate had been detected in all different types of urine samples. Nitrite was not found in normal urine but it had been detected in the urine of* 3 *out of* 10 *bilharzial patients.* 7 *out of* 15 *bladder cancer patients and* 1 *out of* 14 *non-bladder cancer patients.*

INTRODUCTION

THE RELATION between bilharzial infestation and bladder cancer among Egyptian farmers is almost established. However, the nature of the carcinogen(s) and the mechanism of carcinogenesis of the bilharzial bladder cancer is not yet known [1].

Bacterial infection of the urinary tract complicating bilharziasis is extremely common among Egyptian farmers. Unless bilharziasis is treated early, superimposed infection usually follows especially if calculi in the urinary tract are present. Cases of carcinoma of the bladder following bilharziasis are often associated with pyuria bacterial infection and usually precedes the development of malignancy [2]. It has been reported [3] that certain types of bacteria are capable of synthesizing nitrosamines, a well known potent carcinogen [4, 5], from nitrate and amines. This class of compounds can be formed also through chemical reaction of nitrite with amines in acidic medium. Thiocyanate and halogen ions catalize this type of reaction [6].

Nitrate is present in normal urine from diet and drinking water. Varying amounts of secondary amines, produced by the action of bacteria on the digested food in the intestine [7] are also present in urine. Therefore, bladder infected with bacteria might be a suitable site for nitrosamines formation [3].

The aim of the present work is to detect nitrosamines and their precursors in the urine of normal Egyptian subjects, bilharzial infested bilharzial bladder cancer and other types of cancer patients, also to investigate the optimal conditions of nitrosamine formation in urine. A preliminary report had been previously published [8].

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MATERIALS AND METHODS

Determination of nitrosamines

Two hundred millilitres were extracted exhaustively with dichloromethane (DCM). DCM extracts were dehydrated overnight with anhydrous sodium sulfate then concentrated to 3 ml at 40°C. Total nitrosamines were determined using Griess reagent after decomposition with 30% hydrobromic acid in glacial acetic acid according to Eisenbrand and Pruessmann method [9].

Chemical nitrosation of urine

One hundred millilitres of urine were adjusted to pH 4 with HCl, unless it had been stated and incubated in dark brown bottles at 37°C in the presence of 1000 ppm sodium nitrite. After 5 hr the reaction was stopped by adjusting the medium to pH 9.0 with 1 N NaOH. Total nitrosamines were extracted and determined as mentioned above.

Determination of nitrite

Nitrite was determined according to Montgomery and Dymock method [10] using 1% sulfanilic acid in 30% glacial acetic acid and 0.1% *N*-1-naphthyl-ethylene diamine dihydrochloride in 30% glacial acetic acid. Optical density was measured after 10 min at 535 nm.

Determination of thiocyanate

Thiocyanate was determined according to Powell method [11] using 5% ferric nitrate in 2.5% nitric acid. The colour was measured after 5 min at 540 nm.

RESULTS

Tables 1–4 show the analytical data for all urine samples. The mean pH value of different groups was almost identical (range 5.4–7.5). The thiocyanate content was identical in normal and non-bladder cancer with a mean value of 21 and 24 ppm respectively (range 9–56). However, higher values were observed in bilharzial infested and bladder cancer patients, 34 and 39 ppm respectively (range 10–88). Nitrite was not detected in normal urine and in one out of 14 non-bladder cancer urine. On the other hand in bilharzial infested and bladder cancer urine nitrite was detected in 3 out of 10 and 7 out

Table 1. Urine analysis of normal subjects

Case No.	pH	SCN^- (ppm)	NO_2^- (ppm)	Nitrosamine $g \times 10^{-9}/l$ Endogenous*	Nitrosamine $g \times 10^{-9}/l$ After nitrosation†
1	5.6	18	ND‡	ND	23.3
2	5.5	16	ND	ND	6.7
3	4.5	15	ND	ND	93.3
4	5.5	15	ND	16.6	110.0
5	5.4	12	ND	ND	ND
6	5.5	30	ND	ND	3.3
7	6.1	44	ND	16.6	13.3
8	—	—	ND	ND	56.3
9	—	—	ND	ND	199.6
10	—	—	ND	ND	ND
11	—	—	ND	ND	ND
12	—	—	ND	ND	ND
13	—	—	ND	ND	ND
Mean§	5.4	21	ND	16.6	63.2
%‖	—	100	0.0	15.0	61.5

*Total nitrosamine extracted with dichloromethane, estimated by Eisenbrand and Preussmann method.
†Chemical nitrosation was performed at pH 4 in the presence of 1000 ppm sodium nitrite.
‡Not detected.
§Mean of positive cases.
‖Percentage of positive cases.

Table 2. Urine analysis of bilharzial infested patients

Case No.	pH	SCN^- (ppm)	NO_2^- (ppm)	Nitrosamines $g \times 10^{-9}/l$ Endogenous	Nitrosamines $g \times 10^{-9}/l$ After nitrosation
1	6.1	12	ND	10	1157
2	5.3	41	ND	ND	2897
3	5.8	14	5	ND	3667
4	5.4	21	ND	ND	1400
5	5.5	79	ND	10	1930
6	5.8	24	ND	ND	1253
7	5.6	35	ND	ND	2940
8	5.6	6	20	96.7	2703
9	5.3	21	5	ND	1447
10	6.7	88	ND	ND	1640
Mean	5.7	34.1	10.0	38.9	2103.4
%	—	100.0	30.0	30.0	100.0

Legends as Table 1.

Table 3. Urine analysis of bilharzial bladder cancer patients

Case No.	pH	SCN^- (ppm)	NO_2^- (ppm)	Nitrosamine g × 10^{-9}/l	
				Endogenous	After nitrosation
1	5.5	10	ND	ND	1263
2	5.5	30	ND	ND	3003
3	5.6	10	40	6.7	346
4	5.5	21	5	3.4	67
5	5.6	82	ND	ND	2070
6	6.0	47	40	ND	367
7	5.5	38	ND	ND	1297
8	5.9	18	1	3.4	47
9	5.9	88	ND	16.7	183
10	6.0	18	4	3.4	2170
11	5.5	27	ND	ND	2413
12	6.1	87	15	6.7	2123
13	5.9	40	ND	10.0	4523
14	5.5	26	12	13.3	3350
15	6.6	47	ND	ND	6270
16	—	—	—	52.0	8080
17	—	—	—	107.0	6003
18	—	—	—	2.0	1386
19	—	—	—	4.0	215
20	—	—	—	4.0	1343
21	—	—	—	ND	332
22	—	—	—	24.0	1709
23	—	—	—	3.0	7303
24	—	—	—	ND	716
25	—	—	—	ND	1280
Mean	5.8	39.3	16.7	17.3	2314.4
%	—	100.0	46.7	60.0	100.0

Legends as Table 1.

Table 4. Urine analysis of cancer patients other than bladder

Case No.	pH	SCN^- (ppm)	NO_2^- (ppm)	Nitrosamines g × 10^{-9}/l	
				Endogenous	After nitrosation
1	6.3	10	12	ND	1033
2	6.5	24	ND	ND	96
3	5.6	18	ND	ND	57
4	6.8	32	ND	3.3	790
5	5.5	9	ND	ND	111
6	7.5	15	ND	10	280
7	5.5	26	ND	136	657
8	5.0	32	ND	10	910
9	5.5	24	ND	10	720
10	5.4	25	ND	ND	ND
11	6.1	34	ND	ND	60
12	5.6	22	ND	6.7	6
13	5.9	56	ND	ND	ND
14	6.4	13	ND	ND	3
Mean	6.0	24.3	12	29.3	393.0
%	—	100.0	7.1	42.9	85.7

Legend as Table 1.

of 15 cases respectively. Endogenous nitrosamines were detected in some cases of all types of urine in a level of $g \times 10^9/l$ urine, 2 out of 13 normal cases, 3 out of 10 bilharzial patients, 15 out of 25 bladder cancer cases and in 6 out of 14 cases non-bladder cancer.

However, striking differences were observed in the level of total nitrosamines after chemical nitrosation of urine at pH 4 in the presence of excess nitrite ions (1000 ppm). A mean value of total extractable nitrosamines from the urine of bilharzial infested and bladder cancer patients amounts to 2103 and 2314 $g \times 10^9/l$ respectively compared with 63.2 and 393 $g \times 10^{-9}/l$ in the urine of normal and other types of cancer respectively.

Chemical nitrosation is found to be dependent on pH of the urine. As shown in Fig. 1, pH 4 is optimal for chemical nitrosation of urine. In case of bladder cancer urine, nitrosamines could be also formed at pH 7 which is not the case with the urine of other subjects.

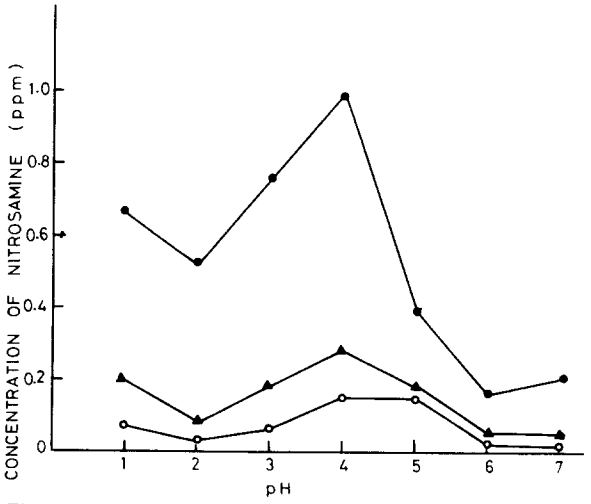

Fig. 1. Effect of pH on the rate of chemical nitrosation of urine of Egyptian normal subjects, bilharzial infested and bladder cancer patients. ○—○ Normal urine, ▲--▲ bilharzial urine, ●—● bladder cancer urine.

DISCUSSION

The ease of formation of nitrosamine from precursors abundant in our food and environment makes nitrosamines a major health hazard to man [4]. More attention had been focused on this subject after confirming that nitrosamines could be also formed *in vivo* in any site where nitrite, secondary amines or nitrate and nitrite-reducing bacteria occur together [3]. It was therefore, of interest to investigate the possibility of nitrosamine formation in the urine of bilharzial infested and bladder cancer patients, which are usually associated with bacterial infection, and to correlate this finding with the high incidence of bladder cancer among Egyptian farmers. A preliminary report had been previously published [8].

The results so far obtained did not show positive correlation between nitrite content of the urine and endogenous *N*-nitrosamine level. These results agreed with our previously published data [8]. However, it is worth mentioning that the amount of nitrosamine extracted with dichloromethane from chemically nitrosated urine of bilharzial and bladder cancer patients is much higher than that extracted from urine of normal subjects (70 times). This indicates that there is greater possibility for nitrosamine formation in the former cases due to presence of high level of amines. The fact that higher levels of endogenous nitrosamines could not be detected in these urines may be due to rapid absorption of nitrosamines from the bladder wall [3] or the presence of insufficient nitrate in the urine at that particular time since all samples were collected from in-patients drinking tap-water usually containing lower nitrate than that found in canal water used by the farmers in the villages. Also it may be explained by the interaction or adsorption of the formed nitrosamines with cell debris and necrotic tissues present usually in the urine of bilharzial and bladder cancer patients.

The thiocyanate content of urine from bilharzial and bladder cancer is higher than that from normal urine but it is not expected that thiocyanate will play a significant catalytic action of nitrosamines formation at pH 5–7, the pH of the urine [6].

Although the acidity of the urine (pH 5.4–7.5) is not optimal for chemical nitrosation (pH 4.0), still bacterial nitrosation could occur. Therefore, it could be expected that urinary bacterial infection was associated with increasing the amount of nitrosamines that could be formed in the bladder, as suggested by the formation of nitrosamines at pH 7 in case of urine of bladder cancer patients (Fig. 1).

The dependence of nitrosamine formation, either by bacterial action or chemical reaction, on the level of nitrite or nitrate ions in urine suggest the importance of investigating the level of nitrate in the diet and drinking water of Egyptian farmers in different areas in Egypt. With the same importance, identification of bacterial types and their capability to catalize nitrosation must be performed. The results of these investigations will be published elsewhere.

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