Afr. J. Biomed. Res. (2000): Vol 3; 129 - 132

AJBR
http:// www.ajbr98.com

Original article

FORMATION OF TOXIC NITROSAMINE AS A COMPLICATION OF PROSTATIC HYPERPLASIA ASSOCIATED WITH URINARY TRACT INFECTION AND URINARY RETENTION.

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The formation of urinary dimethylinitrosamine [DMN] in male patients with benign prostatic hyperplasia [BPH] associated with urinary Tract infection [UTI] was investigated in patients presenting with Klebsiella species infected bladder secondary to [UR]. Specimens from the patients were analysed qualitatively and quantitatively for DMN while blood samples from the same patients were investigated for the activities of common liver enzymes such as alanine transaminase [ALT] and alkaline phosphatase [ALP]. Blood analysis for bilirubin, albumin, total protein content and for creatinine were also carried out. The results obtained in the infected subjects (when compared with those of a healthy group) showed significant mean values of DMN, ALP, AST as 0.12± 0.09mgNO_2/L; 36 ± 1.7iu/L; 7i.u/L respectively (P < 0.05). The values obtained for bilirubin, albumin, total protein, creatinine and ALT were not significantly different from those of control subjects.

Key words: Dimethylnitrosamine, Hepatotoxicity, , Klebsiella species Urinary, tract infection, Urinary retention.

A number of workers [Ashton, 1970; Alaim *et al*, 1971; Ayanaba and Alexander, 1973; 1974; Archer and Stitch, 1983] have reported that the N-nitrosation reaction [the introduction of a nitroso function unto a secondary amine molecule] occurs in the human bladder and the lower primates. This reaction occurs in the presence of bacteria and a precursor secondary amine such as dimethyllamine [DMA], oxytetracycline and amino antipyrine, to form various types of nitroso compounds. The possibility of the interaction, and its attendant toxicity to the liver as a complication in some established cases of urinary tract infection [UTI], was the main focus of the study. This toxicity and its specificity for the liver, as a consequence of the urinary retention and UTI associated with prostatic hyperplasia [PH] is of great concern because of the increased rate of PH especially in the middle aged. More than 75% of such cases develop secondary tract infection UTI and urinary retention [UR]

This study is therefore aimed at establishing a possible link between the formation of the toxic compound nitrosamine. The possible effect on the secretory function of the liver as an underlying factor in cases of prostatic hyperplasia is also highlighted.

MATERIALS AND METHODS

Twenty male patients [ages between 35 and 55 years] attending the University College Hospital Urology [surgical] clinic were used as subjects. The selection criteria include benign prostate hyperplasiap associated with urinary tract infection [UTI] and urinary retention (UR).

The former was confirmed clinically and urine excretion was ensured through the use of appropriate catether. The latter was confirmed by the demonstration of a pathogenic species of Klebsiella in urine voided into sterile containers as well as by the characteristic biochemical reaction of Klebsiella species in a gel [Cheesbrough, 1994]. Aliquots of the test urine from all patients were then analysed qualitatively by thin layer chromatography using analytical Dimethylnitrosamine [DMN] as reference, and quantitatively by a spectrophotometric method [Montgomery and Dyanock, 1961] using a standard calibration curve for its conversion via quantitative nitrite producing degradation of DMN by ultraviolet irradiation.

Blood specimens were also collected from the twenty patients into Lithium heparin bottles and analysed for the activities of alkaline phosphatase [ALP] alanine and aspartate transaminases [ALT and AST] and for conjugated and uncojugated bilirubin, total protein and albumin concentrations as indicators of liver toxicity. Creatinine levels of the same blood specimens were determined to establish the functional state of the kidney. Blood and urine specimens were also collected from twenty healthy subjects aged between 25-52 years consisting of employees and students in the hospital, these served as the controls. Creatinine levels of the same blood specimens in 20 healthy subjects were also determined.

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Microbiological analysis for Klebsiella species was carried out as follows: - Urine samples from both control and test subjects were examined macroscopically for red blood cells pus cells epithelial cells, crystals or casts which are secondary indicators of urinary tract infection [Cheesbrough, 1994] a neat portion of each urine specimen after mixing was later cultured aseptically on both MacConkey and CLED Agars [Cheesbrough, 1994] and incubated at 37^{0}C overnight. Klebsiella colonies with their typical shinning appearance and shapes [Cheesbrough, 1994] were later subcultured into Fimmon's citrate medium [Cheesbrough, 1994] and further reconfirmed using indole reagent.

Qualitative analysis of the urine (Qualitative test) for the presence of DMN.

Thin layer qualitative analysis of the urine specimen from patient and control group was done to establish the presence of DMN using SILICAL-GEL [Type G MFP] on glass plates. N-hexane-ether-dichloromethane solvent mixture [4:3::2:2] was used as the solvent system and separation allowed to proceed for 6 hours at room temperature [28^{0}C]. Pure standard DMN was included in the run as Reference. The plates were then removed after separation. The migrated spots were marked and then sprayed with each of the two reagents (Palladium II chloride/ diphenylamine and Sulphanilic acid + 1-naphthylamine) as recommended by Preussmann *et al* (1964) on separate T.L.C. plates. Positive purple colour for the reagents confirmed the presence of nitrosamine.

Biochemicals Test For Organotoxicity

Alkaline Phosphatase assay: Alkaline phosphatase Assay was done on both the subjects and control blood plasma using the modified kinetic method of Bowers and McComb [1972] incorporating appropriate serum and reagent controls.

Alanine and Aspartate Amino Transferase assay.: ALT and AST activities were measured in the plasma of both the subjects and control plasma using the modified kinetic method of karmen *et al.* [1955] incorporating appropriate serum and reagent controls.

Conjugated and Unconjugated billirubin assay: The assay was carried out in both subject and control samples using the modified method of Henry *et al* [1953] with appropriate controls.

Plasma total protein and albumin assay: Total protein and albumin concentrations were measured using the standard Biuret and Dye binding [Bromo Cresol green] techniques [Kingsley, 1972] respectively.

Plasma Creatinine concentration: This was measured using the alkaline picrate method of Jaffe and Larsen [1972].

Statistical Analysis of the data

The data obtained for ALT, AST, ALP, total protein and albumin determinations were subjected to statistical analysis using the student t-test while the statistical significance for the t-test was assessed using a two-tailed probability level of 0.05.

RESULTS

Establishment of bacterial infection of the bladder

Klebsiella species was established as pathogenic bacteria in the Urinary tract of the subjects by identifying the organism by their typical characteristic colonies. This was further confirmed through classical biochemical reactions of the organism on sugars and with indole reagents. This sharply contrasted with the results obtained from urine cultures of control group where no organism was isolated (Table 1).

Dimethylnitrosamine [DMN] formation : Quantitative analysis showed that the mean DMN formed in the Urine specimens of the control group was $0.15 \pm 0.09 mgN0_2$- N/L as against those of subjects with infected bladder having a mean of $0.28 \pm 0.09 mgN0_2$-N/L, $P < 0.05$ (Table 2)

Tests indicating Liver toxicity.

The results of blood plasma specimens as analysed for ALP, ALT, AST, billirubin, total protein and albumin are shown in Tables 2 and 3. The mean ALP was found to be 71 i.u/L in the infected group as against that of healthy control which was 35iu/L. The mean ALT, and AST activities were 11 i.u/L and 23 i.u/L respectively for the infected group. The mean plasma bilirubin, total protein and albumin concentrations were also found to be 0.4mg/100ml; 3.95g/100ml respectively for the infected subjects as compared correspondingly with 0.6mh/100ml; 7.5g/100ml; ans3.9/100ml values in the healthy control group.

Statistical analysis showed that P-values were 0.79, 0.82, and 0.72 for total protein, albumin and billirubin respectively, indicating that the variations were non-significant [i.e P > 0.05]. However, P-values for ALP, ALT, AST were found to be 0.059, 0.0025 and 0.00025 respectively [i.e P<0.05] indicating that the variation obtained for the two Enzymes- ALP and AST in the control and subject groups were statistically significant.

TABLE 1
Microbial profile of urine cultures of patients with benign prostatic hyperplasia [BPH]

Patients' No	**Pub. Cells**	**Red Blood Cells**	**Epith. Cells**	**Crystals**	**Cast**	**Organism isolated**	**Inference**
1	++	+	++	+++ (P)	-	Klebsiella species	Klebsiella species infected UTI
2	++	++	++	++ (P, OX)	+	"	"
3	++	++	+	(P)	+	"	"
4	++	+	-	-	-	"	"
5	+++	+++	++	(P, OX)	-	"	"
6	+++	+++	_	(OX)	-	"	"
7	++	+++	+	+ (P)	++	"	"
8	++	+++	++	+ (P)	+++	"	"
9	++	+	+	++ (P)	-	"	"
10	+++	++	+	++(OX)	+	"	"
11	++	++	+	+ (OX)	-	"	"
12	++	+++	+	+ (OX)	+	"	"
13	+++	+++	+	+ (OX)	+	"	"
14	+++	++	+	+ (P)	+	"	"
15	++	+	+	+ (P, OX)	-	"	"
16	++	++	++	++ (P)	++	"	"
17	++	++	++	+ (P)	+	"	"
18	+++	++	++	++ (P, OX)	-	"	"
19	++	+	+	+ ()X)	+	"	"
20	+++	++	++	+ (P)	+	"	"

TABLE 2
Effect of benign prostatic hyperplasia [BPH] on DMN, Alkaline Phosphatase [ALP], Alanine amino transferase [ALT] and Aspartata amino transferase [AST] values.

	DMN Concentrations [mgNO_2^-/L)		ALP (i.u/L)	ALT [i.u./L]	AST [i.u./L]
	Before u.v irradiation	After u.v. irradiation			
Control	**0.15 ± 0.09**	**0.16 ± 0.12**	**35 ± 17**	**16 ± 5**	**16 ± 5**
Patients with BPH	**0.16 ± 0.10**	**0.28 ± 0.09**	**71 ± 38**	**11 ± 4**	**23 ± 6**
P value	**< 0.5**	**> 0.5**	**0.05**	**0.0025**	**.00025**

DISCUSSIONS

The toxicity and carcinogenicity of N-nitrosamine in a large number of organs in different species of animals have long been established [Magee and Bames, 1959]. Its potency as a chemical carcinogen which was later demonstrated to be related to its degree of chemical substitution of the nitrosamine molecule was yet another pointer to the danger posed by this group of compounds. [Lai, 1980]

However, the occurrence of this group of chemicals as pollutants of the environment especially the human work place [Lijinsky and Epstein, 1970] was not of much interest until nitrosamines were discovered to be formed naturally within the human environment [Fan *et al*, 1977] In addition, the compound was demonstrated in the vaginal vault of humans [Nun *et al*, 1974] and later in beverages and alcoholic drinks commonly consumed in the tropics by humans [Maduagwu, 1976].

The revelation that nitrosamine could be formed from some of the precursor compounds-nitrates and amines, under appropriate condit6ions, such as the presence of bacteria through nitrosation reaction in the human bladder [Hill and Hawksworth, 1972; Mirvish, 1977] is of much concern partly due to the high incidence of UTI with UR [Ashton, 1970] and abundance of the nitrates and amines in the common vegetables in this environment [White, 1975]. However, in this study, very

small quantities of one of the most potent nitrosamine (DMN) was shown to be present in the bladder of the patients investigated in comparison to the control group.

TABLE 3
Plasma billirubin, total protein, albumin and creatinine in control subjects and patients with benign prostatic hyperplasia [BPH]

	Billirubin [mg/dl]	Total protein [g/dl]	Albumin [g/dl]
Control	**0.6 ± 0.3**	**7.5 ± 0.4**	**3.9 ± 0.5**
Patients with BHP	**0.4 ±S 0.2$^{N.S}$**	**7.4 ± 0.8$^{N.S}$**	**3.9 ± 0.4$^{N.S}$**

Although, the alterations produced in plasma enzyme levels of the subjects are minimal, the fact that the ALP, ALT and AST concentrations are significantly increased compared to the control is a sufficient pointer to the fact that DMN formation could be a complicating factor in the incidence of prostatic hyperplasia, a percentage of which usually end in urinary tract infection and urinary retention.

This possibility cannot be overemphasised especially with such prevailing environmental conditions such as massive bacterial infections of the bladder, the vegetarian dietary habit of the populace and the lack of proper environmental protection in the community. The formation of any of the toxic nitroso compounds can therefore not be overlooked as a possible complication in this condition. However, to be able to reasonably justify this speculation, more work in the given directions need to be accomplished.

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Received: 10th April 1999
Accepted in final form: 20th August 2000