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EVALUATION OF MUTAGENICITY RISK AMONG NURSES EXPOSED TO CYTOSTATIC IN A UNIVERSITY HOSPITAL IN EASTERN OF ALGERIA

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Ames test, occupational exposure, cytostatic

Abstract

Aims: The aim of this study is to assess the mutagenicity effect of occupational exposure to cytostatc in order to propose an environmental medical prevention program.

Materials and Methods: A cross-sectional descriptive study was conducted for three months, it involved 39 nurses exposed to cytostatic drugs in oncology and 43 referents. They were matched by age, sex, smoking, seniority .The data were collected using a medical questionnaire. Mutagenicity was evaluated by the Ames test. The calculation of the ICC has allowed the classification of exposure.

Results: The average age of exposed subjects is 42.28 ± 9.90 years with female predominance. 75% of staff is classified exposure level 3 and 2.

Among the contact with cytostatic drugs is associated with a risk of mutagenic 3 (RR = 3.28, 95% CI: 1.73, 6, 22) with a highly significant difference (P << 0 .0001). 60% of samples of exposed and 15% of referents were positive, the mean number of positive revertants was 527.94 with a good correlation between the number of revertants and the ICC.

Conclusion: This study confirms mutagenic risk among nurses handling cytostatic without adequate safeguards. It encourages the alignment on best practices to improve working conditions in oncology structures.

Introduction

The first study showing evidence of genotoxicity in the personal care handling cytostatic was conducted on urine of nurses involved in the administration of chemotherapy in a Finnish oncology service using the Ames test [1].

And for over thirty studies were conducted. The majority of articles were published before the nineties. The results are controversial, in connection with numerous methodological problems (early urine collection after short exposure to drugs, drug intake, confounding factors, etc.) [2, 3, 4, 5, 6] .Divers authors recorded increased urine mutagenicity power among nurses handling these drugs without means of protection [1, 2.7, 8.9] and / or inappropriate. [10] Conversely, other studies have not revealed positive mutagenic activity [10,11,12,13,] .In certain situations, the interpretation of results is difficult and does not conclude with certainty the presence or absence of mutagenic activity (doubtful test) [14,15,16,17].

Since the publication of best practices for preparation and administration of cytostatic, in several countries, this test was used to assess the effectiveness of preventive measures in particular the preparation in vertical or horizontal laminar flow hood. No mutagenic urine was highlighted for preparation of chemotherapy in vertical flow hood, conversely to the hood horizontal laminar flow. [18] The mutagenic activity of urine has been proposed by some authors for monitoring workers exposed to cytostatic. [19]

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Our study aims was to assess the mutagenic risk among nurses exposed to cytostatic to outline an environmental medical prevention program.

Materials And Methods

A cross-sectional descriptive analytic study was conducted for three months from November 2014 to January 2015. It concerned 82 subjects: 39 nurses exposed to cytostatic of University Hospital center in eastern Algeria and 43 referents. The two groups were matched for age, sex, smoking and seniority at the workplace.
After their consent, each subject received medical questionnaire containing several topics (demographics and professionals, toxic habits, history, drug intake, power type), a clinical examination and a urine collection for Ames test.

Evaluation of mutagenicity

The evaluation of mutagenic cytostatic power was made by the Ames test. We used the TA 102 strain derived from Salmonella typhimurium LT2 strain carries a mutation in a gene coding for the synthesis of the amino acid histidine, this mutation renders the bacteria unable to grow on medium without histidine . The strain was stored at -80 ° C and regularly checked for genetic markers (tetracycline resistance, rfa mutation and UV sensitivity) .

Protocol Ames test

From a preserved strain, 20 µl of the strain are grown in 5 to 20 ml of nutrient broth supplemented with a histidine and traces of biotin and 25 g / ml of tetracycline. After 18 hours of incubation at 37 ° C in a shaking water bath, 100 .mu.l of this culture are contacted with 100 µl of each dilution of the test molecule, and 500µl of buffer. The mixture is incubated at 37 ° C for 20 min. Two milliliters of top agar (0.6% agar and 0.6% NaCl) are added to the mixtures and paid after gentle agitation, on the minimum agar (constitutes 1% of MgSO4, 10% citric acid, 50% of K2HPO4, 17.5% of Na2NH2PO4-4H2O, 2% glucose, and 1.5% agar). After solidification of the mixture, the dishes are incubated at 37 ° C for 48 to 72 hours. Once the cans are removed from the incubator, colonies are counted and the results are expressed in the form of revertant colonies per plate [20].

Reading and interpretation of results: the visual counting is easy, unlike the stumps by number plate is less than 100, for strain TA 102; an increase of twice can be observed (more than 200) .The result of the Ames test is considered positive if the concentration tested for the number of revertants induced is equal to or greater than twice the number of spontaneous revertants. The validity of the test is ensured by the positive response obtained with the positive controls [21].

Assessment of exposure to cytostatic

To assess the importance of contact with cytostatic drugs, we calculated the Index Contact Cytostatic (ICC), only parameter that currently evaluating simply exposure. It was calculated over a period of 15 days from the following formula:

ICC = n R + nA / Nh

- nR: number of preparations carried out by a person in a given period
- nA: number of administrations carried out by the same person for a specified period.
- nH: number of hours of presence of this person during the specified period.

This index identifies three levels which are attached special precautions:

- Level 1: ICC <1: preparation and administration occasionally, need for individual prevention.
- Level 2: 1 <ICC <3: preparation and administration in moderate amounts, need for establishment of a collective prevention (security post cytostatic).
- Level 3: ICC> 3: intensive preparation and administration, establishment of a centralized reconstruction unit.

Statistic Study

Data were entered and analyzed by software XL STAT 2015 and Mini Tab 16.
Employees' statistical tests for data analysis were:

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- Comparison tests: t-test (comparison of two averages) and the z-test (comparison of proportions) and Anova
- The association test: calculation of the relative risk (RR).

P-value < 0.05 was considered statistically significant

Results

Sociodemographic and professional characteristics of the study population

There is no significant difference between the exposed group and the group referred the matter concerning sociodemographic characteristics (age, sex, educational level and marital status) and professional (seniority). The exposed group consisted of 39 nursing relatively young with an average age 42.28 ± 9.90 years, female predominance (sex ratio = 0.21), the level of secondary education (80%) and marital status "married.

Calculation of the index contact cytostatic

The medical oncology service was classified as level 3, while that of hematology was classified as Level 2. The pediatric oncology was classified as Level 1 (table 1).

Table 1: Comparison of the ICC oncology structures (2014-2015)

	Level	**Average**	**Standard Deviation**	**P-value**
Oncology	3	8,83	1,47	0,000***
Hematology	2	1,54	0,30	
Pediatric	1	0,50	0,28	

Study of mutagenicity Ames test

Two techniques were used for the interpretation of Ames test: the comparison with the negative control and the calculation of the average positive revertants in exposed and non-exposed. The results are similar for both methods, with a very highly significant difference.

The Ames test was positive for 24 nurses exposed to cytostatic, versus, 15 unexposed. The relative risk of 3.28 (95% CI: 1.73, 6, 22), the difference was highly significant (P << 0 .0001). (Table 2)

Table 2 :Association between exposure to cytostatic in a University Hospital centre in Eastern Algerian and positivity the Ames test (2014-2015)

	Exposed = 39	Referents= 43	RR	IC 95%	P-value
Ames test (+)	24	15	3,28	(1,73; 6,22)	0,000***

For the first method, 60% of samples of exposed subjects and 15% of non-exposed were positive (Table 3).

For the second method, the average number of revertants was 527.94 ± 197.73 for exposed and 297.17 ± 90.34 for referents (Table 2).

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Table 3: Comparison of results of the Ames test between exposed to cytostatic and unexposed (2014-2015)

Revertants +	Minimum	Maximum	Average	SD	P-value
Exposed	200.00	1000.00	527.94	197.73	0,000***
Referents	199.00	490.00	297.17	90.34	
Test	**Positive**		**Negative**		**P**
	N **%**		**N** **%**		
Exposed	24	60 %	15	40 %	0,000***
Referents	9	15%	34	85 %	

The distribution of results of mutagenicity by the level of CCI shows an association between the number of revertant and the ICC. The number of revertants increases with the number of preparations and administration of cytostatic drugs. It increased to 378.75 for level 1 to level 2 388.33 to 597.30 for level 3. This increase was highly statistically highly significant (p <0.001) (Table 3).

Table 3: Breakdown of the Ames test results in exposed to cytostatic by level of ICC (2014-2015)

	ICC 3	ICC0	ICC 1	ICC2
Minimum	230.00	199.00	230.00	200.00
Maximum	1000.00	490.00	490.00	480.00
4 th Quartile	460.00	240.00	357.50	325.00
Median	560.00	262.50	385.00	435.00
3rd Quartile	723.00	317.75	422.50	477.50
Average	**597.30**	**297.17**	**378.75**	**388.33**
Variance	38761.21	7958.34	5360.93	11147.22
Standard deviation	200.77	90.34	78.27	115.65
Standard deviation for the Average	39.37	14.28	27.67	47.21
Lower bound average (95%)	516.21	268.28	313.26	266.94
upper bound average (95%)	678.40	326.06	444.23	509.71

Anova F = 25,26 P= 0,000***

Discussion

Nurses are exposed in the structures of Oncology to cytostatic during the preparation and administration of chemotherapy drugs. Sometimes, the exhibition is made after contact with excreta and vomit of patients.

The study of working conditions has revealed the lack of separation between local preparation and administration, the importance of the workload (on average 70 procedures per day in the medical oncology service), the lack of means of collective protection (no hood or insulator) and inadequate personal protective equipment which were limited to gowns and gloves. Latex gloves are worn only by 26% of nurses. The workload was confirmed by calculating the ICC or 75% of nurses belonged to level 2 and 3 services.

It is clear from our work that the risk of having a positive Ames test is three times higher in subjects exposed to cytostatic (RR = 3.28, 95% CI: 1.73, 6, 22)
Sixty percent (60%) samples of exposed subjects, versus 15% of un exposed were mutagenic. Our results coincide with those published in 2013 by Allaire who reported a positivity rate of referents in Ames test 10%. [22]

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The results of our study converge to a strong association between exposure to cytostatic and positivity the Ames test. The average revertants is very high, it is 597.30 ± 200.77 with a parallel increase with the level of exposure to cytostatic. This result is explained by the importance of the workload, the lack of specific means of collective and individual protection and the number of drugs to prepare. Staff handle 9 mutagenic, 7 drugs are very dangerous (ifosfamide, gemcitabine, Camto, cyclophosphamide, dacarbazine, carboplatin, adriamycin), and two dangerous (Eloxatin, Bleomycin). Among the cytostatic, it is mainly alkylating substances with mutagenic and carcinogenic action, exposure index cyclophosphamide is 1.

In our series, we recorded a highly significant difference; the same findings were reported in the literature to nurses and pharmacists preparing cytostatic unprotected. In a typical study exposed / unexposed, Benhamou evaluated the mutagenic cytostatic power by comparing 29 nurses preparing cytostatic drugs without special precautions and 29 unexposed. The Ames test was positive with a statistically significant difference [2]. The same result was found by Pohlová et al studying the mutagenic cytostatic power in 39 exposed and 19 referents [23].

Similarly, Caudel et al in 1988, found significant results when performing the Ames test in nursing oncology services [8]. Identical findings were published by Clonfero et al (1989), Falck et al (1979) [9,1].

Similar results were observed in the manipulators cytostatic drugs, but without specifying the working conditions and the availability and wearing of protective equipment (Table) Roth (1995), Newman (1995) [23, 24]..

Other authors found a urine mutagenicity of workers handling cytostatic under horizontal laminar flow hood. Thiringer (1991), reported the positivity of the Ames test in 60 nurses preparing courses of chemotherapy under a laminar flow hood [19]. It is the same for Anderson's conclusions in 1982 [18].

In the series of Kolmodin-Hedman , work under a hood and wearing gloves were insufficient to protect nursing mutagenic hazard cytostatic [10],

Conversely our conclusions in some work, the mutagenic cytostatic power has not been confirmed (Table 4)

Table 4: Study of mutagenicity of the urine of workers exposed to cytostatic

Authors	Population	Working Conditions	Results	Ref
Rezaei(2013)	Nurses 138	Pas de précision	Positive	25
Labuhn (1998)	Nurses Pharmacists 83/35	No particular precaution	Doubtful	17
Demeo (1995)	Nurses 38/25		Doubtfull	35
Roth (1995)	Nurses	Unspecified	Positive	23
Newman (1994)	Nurses 24/25	Unspecified	Positive	24
Guinée EP (1991)	Pharmacists 15/20	Unspecified	Negative	26
Thiringer (1991)	Nurses 60/60	HFL	Positive	19
Krepinsky (1990)	Nurses	Unspecified	Negative	27
Elliot (1990)	Pharmacists 6	Unspecified	Negative	28
Clonfero (1989)	Nurses 9/11	No particular precaution	Positive, SD	9
Rossner (1988)	Preparers 38/18	Unspecified	Positive	29
Caudell (1988)	Nurses	No particular precaution	Positive, SD	8
Poyen (1988)	Nurses 47/37	HFL vertical	Negative	4

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Stucker (1986)	Nurses	No particular precaution	Positive, SD	7
Pohlova (1986)	Preparers Chimists 38/19	No particular precaution	Positive	30
Friederich(1986)	Nurses 24/	No particular precaution	Doubtful	15
Benhamou (1986)	Nurses 29/29	No particular precaution	Positive, SD	2
Barale (1985)	Nurses 21/21	Unspecified	negative	31
Everson (1985)	Nurses Pharmacists 26/38	LFH approprate protection	Negative	3
Venitt (1984)	Nurses	LFH horizontal	Negative	32
Ratcliffe (1983)	Nurses	LFH vertical	Negative	33
Kolmodinhedman(1983)	Nurses	Hood and gloves	Positive, SD	10
Anderson (1982)	Nurses Pharmacists 6	LFH vertical, LFH horizontal	Positive	18
Bos (1982)	Nurses	Unspecified	Doubtful	14
Staiainio (1981)	Nurses	LFH vertical ou horizontal	Negative	34
Falck (1979)	Nurses	No particular precaution	Positive, SD	1

*[Ref]:reference [LFH]: laminar flow hood [SD]: significant difference

Conclusion

This study confirms the mutagenic cytostatic risk in the population of workers handling these drugs in the absence of adequate safeguards. It suggests alignment with best practices for improving working conditions in oncology structures. This is particularly the biannual special medical supervision, centralization of preparations in the URC, the staffing of personal protection means and staff training on occupational hazards. .

No Conflict Of Interest

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