

Evaluation of morbidity risk in school age children living under arsenic influence conditions and significance of micronuclei assays

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ABSTRACT

It has been studied morbidity structure of school age children living in Likhuni gorge under arsenic influence. By comparing with control are documented authentically high frequency of doctoral visits and acute respiratory infections incidence. To confirm the arsenic influence we have determined micronuclei (MN) level in buccal exfoliation cells that reflects genetic changes. It is shown, that MN number correlates with arsenic concentration in environment. Has been reached authentic correlation between MN level and morbidity with acute respiratory infections.

KEYWORDS: *ecology, arsenic, micronuclei, children' health*

There is a pollution of environment by industrial waste in a former arsenic industry territory in the high mountain region of Racha. According to hydrogeologists' data the arsenic concentration in Likhuni gorge' air, water and ground are significantly increased. On the territories distant from Likhuni gorge these indicators are less and on the intact regions and in rivers are approximately normal [4]. Cumulative intoxication by arsenic ions causes the damage of genetic, immunologic and hematologic systems [6,7]. There are data showing increased frequency of acute respiratory infection (ARI) in the mentioned region [1,8,9]. It's known a connection between structure of the diseases and ecological distinction of the area [2,3]. Most environmental agents, including heavy metals, reveal mutagenous traits and can cause genetic changes in human. These changes could be monitored by different cytogenetic methods, among them is calculation of micronuclei number (MN) in the cell [10].

The purpose of the work was to investigate how the arsenic polluted environment influence on the children' and adults morbidity indicators by using MN study method and estimate dimension of the risk factors.

The goals were:

1. Study the structure of school age children morbidity in the regions where is ascertained the different pollution level by arsenic (Likhuni gorge, Ambrolauri and nearby villages);
2. Determine the correlation between morbidity with various diseases and amount of micronuclei in buccal exfoliation cells;
3. Estimation the value of cellular MN amount to prognostication various diseases in school age children.

MATERIALS AND METHODS

Over five -year period (1999-2004) there were under observation 143 school children living in Likhuni gorge _ near the territory of arsenic mining (I group) and 143 same age children living in Ambrolauri and nearby villages (50 km remote from Likhuni gorge) in the same economic conditions and with analogous parameters (II group). The latter group members were under less arsenic influence (randomize control).

Sickness rate calculation was carried out according to international classification X review, taking into

consideration different classes [5]. By using the cohort research we studied existed arsenic environment and its influence on the children and adults morbidity. In the both groups was calculated relative and attributive risk dimensions under influence of arsenic and without it.

Cytogenetic research has been performed in the buccal exfoliation cells according to Stich's method. There were investigated 50 school children from Likhuni gorge (I group), 100 children from Ambrolauri and nearby villages (II group) and Tbilisi inhabitants - 25 healthy school children (control group). In each case there were analysed 500-1000 cells.

We have counted quantity of cells with MN and MN number in them. Results were counted again per 1000 cells.

Outcomes were analyzed statistically. By using case-control method we evaluated risk factors, relative and attributive risk dimensions under influence of arsenic, their reliability interval for each factor according to groups. Comparison of results was performed by the χ^2 criterion (Pearson) for qualitative data and by t - student criterion for quantitative data. Difference was considered as authentic when $\chi^2 > 3,84$, $p < 0,05$; and $t > 1,96$, $p < 0,05$ [12]. For correlation analyses we have used Spirmen's qualitative correlations. Calculations were performed using computer software SPSS 11.5. It was determined the value of exfoliation cell's MN amount to prognostication various diseases.

RESULTS AND DISCUSSION

Among the investigated persons mainly was observed 4 classes of diseases: infectious – parasitic and diseases of respiratory, digestive and skin systems. In all investigated areas the structure of diseases was similar _ it were predominated respiratory system diseases. This was also confirmed by comparing results with 2001-2004 years ones ($t > 2$).

Also was determined frequency of morbidity with various diseases among inhabitants of arsenic influence zone and controlled group's children. It was documented, that in the first group is certainly high doctoral visits number, frequency of acute respiratory infection with multi-localization, other acute infections and thyroidal adenoma. By comparing with control group the frequency of atopic dermatitis, allergic rhinitis, conjunctivitis, acute

obstructive laryngitis, croup (0-1 degree) was approximately authentically grown.

The estimation of the relative and attributive risks of morbidity among Lukhuni gorge inhabitants has shown, that the relative and attributive risk of ARI is high (RR=90,07; AR=0,80; CI-RR=12,78; CI+RR=634,62; CI-AR=0,74; CI+AR=1,49). Six times and more is elevated frequency of doctoral visits (RR=47,32; AR=0,97; CI-RR=15,44; CI=RR=145,03; CI-AR=0,93; CI+AR=1,20) due to acute respiratory infection with multi-localization, other acute infections (RR=51,39; AR=0,66; CI-RR=7,32; CI+RR=360,9; CI-AR=0,59; CI+AR=1,42), acute obstructive laryngitis, croup (0-1 degree), atopic dermatitis, conjunctivitis, suggested infectious diarrhea by origin and gastroenteritis, adenoma of thyroid. All data are statistically authentic and confirmed by I and II groups illness frequencies comparison.

To confirm arsenic's harmful affection, in both groups we have studied MN distribution in buccal exfoliative cells. Control group was composed of 25 healthy school age Tbilisi inhabitants. See research results in the *Tab.1*.

The average quantity of cellular micronuclei in the I group is noticeably high, than in the II and control ones.

We can presume that MN number in the I group was elevated due to arsenic influence, because other environmental factors, including background radiation in investigated territories, are absolutely similar. It was

confirmed correlation between morbidity and MN number. More over, it was revealed statistically reliable correlation between MN quantity in 1000 exfoliation cells and ARI diseases frequency (R=0,789; p<0,000001).

We have compared data of the I group with those children's data from the II group who often are down with respiratory infections. MN average number in 1000 exfoliation cells higher in the I group. Difference is statistically authentic (t=55,3; p<0,0000).

We also have compared the data of the I group with seldom ill children's data from the II group.

The MN's number was higher in the I group. Difference is statistically authentic (t=113,1; p<0,0000). It was determined MN distribution among groups. See the results in the *Tab.2*.

In both groups we have studied relative and attributive risk of MN's average number (2-14) genesis. These risks are low in Lukhuni gorge than in Ambrolauri and nearby villages (RR=0,08; 95%CI=0,03-0,24); But there are high relative and attributive risk of MN high quantity (14-36) genesis (RR=46,67; 95%CI=15,23-143,92).

One of our goals was to determine MN test's sensitivity under arsenic influence for various diseases. MN existence exhibits high correlation with nosologies from the table 3. That allows to consider this test as a sensitive to determine the risk of mentioned diseases onset (*Tab.3*).

	#	Girls	Boys	Minimal number of MN per 1000 exf. cell	Maximal number of MN per 1000 exf. cell	Counted cells number	MN distribution	MN's average number per 1000 exf. cell
Lukhuni gorge	50	22	28	10	36	36123 (820)	2 MN in 16 cells of 8 children, in all other cases 1 per cell	22,38+1,26
Ambrolauri and nearby villages	100	53	47	0	25	29380 (132)	1 in all cases	4,36+0,5
Tbilisi	25	12	13	0	2	10000	Unitary MN in 16 children	0,72+0,12

Tab.1 MN distribution in I, II and control groups.

Factors	Frequency (I group)	Frequency (II group)	χ^2
0-2	0,00	0,02	3,03
MMN	2-14	0,02	63,03
	14-36	0,98	262,50

Tab.2 I and II groups MN average numbers comparison.

<i>Factors</i>		<i>Truly positive a ++</i>	<i>Falsely positive b +-</i>	<i>Falsely negative c -+</i>	<i>Truly negative d --</i>
Allergic rhinitis	abs.	10	3	59	25
	%	10,31	3,09	60,82	25,77
Acute obstruction bronchitis	abs.	17	1	104	28
	%	11,33	0,67	69,33	18,67
Flu and other respire	abs.	14	1	107	28
	%	9,33	0,67	71,33	18,67
Acute respiratory infection with multi-localization, other acute infections	abs.	83	15	38	14
	%	55,33	10,00	25,33	9,33

Tab.3 Characteristics of MN test for different respiratory diseases.

<i>Diseases</i>	<i>Sensitivity</i>	<i>Specificity</i>	<i>Prognostic value of positive results</i>	<i>Prognostic value of negative results</i>	<i>Diagnostic precision</i>
Allergic rhinitis	0,145	0,893	0,769	0,298	0,361
CI ⁻	0,062	0,778	0,540	0,200	0,265
CI ⁺	0,228	1,007	0,998	0,395	0,456
Acute obstruction bronchitis	0,140	0,966	0,944	0,212	0,300
CI ⁻	0,079	0,899	0,839	0,142	0,227
CI ⁺	0,202	1,032	1,050	0,282	0,373
Flu with other respiratory indications, asthma syndrome	0,116	0,966	0,933	0,207	0,280
CI ⁻	0,059	0,899	0,807	0,139	0,208
CI ⁺	0,173	1,032	1,060	0,276	0,352
Acute respiratory infection with multi-localization, other acute infections	0,686	0,483	0,847	0,269	0,647
CI ⁻	0,603	0,301	0,776	0,149	0,570
CI ⁺	0,769	0,665	0,918	0,390	0,723

Tab.4 Estimation of the test's sensitivity.

As it shown MN test has low sensitivity and high specificity for allergic rhinitis, acute obstruction bronchitis and flu with other respiratory indications, asthma syndrome. In the cases of acute respiratory infection with multi-localization, other acute infections test exhibits high sensitivity and middle specificity. For all mentioned diseases the prognostic value of positive results is high and prognostic value of negative results is low.

CONCLUSIONS

Thus under the Arsenic influence MN test is sensitive to elicit this affection and prognosis of ARI onset. It could be concluded that „harmful“ environmental factors, including arsenic, depress children' adaptation abilities. It could explain revealed correlation between MN existence and respiratory illness or other mentioned diseases.

1. Arsenic polluted environment affects the children and adults health, and elevates incidence of respiratory and other diseases;
2. Due to arsenic pollution there is authentically increased MN number in children' and adult buccal exfoliative cells which correlates with pollution level;
3. MN number in exfoliative cells authentically correlates with ARI illness high frequency;
4. Under the arsenic influence MN test is sensitive to elicit this affection and prognosis of ARI onset.

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Риск заболеваемости и значение учета микроядер среди детей школьного возраста, испытывающих воздействие мышьяка не в окружающей среде

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Р Е З Ю М Е

Изучена структура заболеваемости детей школьного возраста в районе Лухунского ущелья, страдающей загрязнением окружающей среды мышьяком. Было зафиксировано (по сравнению с контролем) статистически достоверное увеличение обращаемости к врачу и рост заболеваемости острыми респираторными инфекциями. Для объективного подтверждения воздействия мышьяка на организм детей исследован уровень микроядер (МЯ) в эксфолиативных клетках полости рта, что указывает на существующие цитогенетические изменения. Установлено, что уровень МЯ коррелирует со степенью загрязнения окружающей среды. Выявлена достоверная корреляция между уровнем микроядер и частотой заболеваемости острыми респираторными инфекциями.

Ключевые слова: мышьяк, мутаген, микроядра, здоровье, дети, экологическая ситуация

□ **International committee of medical journal editors. Uniform requirements for manuscripts submitted to biomedical journals.** Ann Intern Med 1997;126:36–47.

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