SOMATIC MUTATION IN PERIPHERAL BLOOD LYMPHOCYTES AMONG METRO MANILA RESIDENTS: INDICATOR OF EXPOSURE TO ENVIRONMENTAL POLLUTION

Ma. Teresa Yulo-Nazarea, Maria Lucia C. Cobar, Marla A. Endriga
*Efren J. Sta. Maria, Alejandro Q. Nato, Jr., Javier Eduardo and *Romy Dy*
Health Physics Researh
Philippine Nuclear Research Institute
Diliman, Quezon City, Philippines
**National Kidney Institute*
Department of Health
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Metro Manila is ranked as one of the world's most polluted cities where air quality levels are 2-3 times higher than the levels set by WHO. Development of diseases could be alleviated if early warning signs as occurrence of gene mutations are detected early enough. The adapted hypoxanthine guanine phosphoribosyl transferase (HGPRT) mutation assay measures the degree of mutation on the HGPRT gene and allows rapid evaluation of the occurrence of mutation in an individual exposed to radiation or mutagens within six months after exposure. The objective of the project is to (1) assay exposure of Metro Manila residents exposed to environmental pollution, (2) determine population groups significantly affected by pollutants and (3) construct an environmental baseline HGPRT mutation data bank specific to area in Metro Manila. A composite table of personal information of donors against mutation index in two barangays in Valenzuela is presented. About 30% of the total samples are shown to have mutation index greater than 0.5. So far, the data show a slightly higher mutation rate among donors who are smokers with more than 5 hours outdoor exposure to pollutants per day than the corresponding class of non-smokers.

INTRODUCTION

Metro Manila is ranked as one of the world's most polluted cities where air quality levels are 2-3 times higher than the levels set by the World Health Organization (WHO). An inventory of pollutants emanating from motor vehicles showed alarming levels of particulate matter, SO_2, CO, NO_2, organic carbon and other known health effects classified as toxic air contaminants (TAC). The total soluble particulates (TSP) level reported in 1994 by the Air Quality Monitoring Service (AQMS) of the Department of Environmental and Natural Resources- National Capital Region (DENR-NCR) showed that Valenzuela registered the highest concentration of TSP in ambient air on a 24 h measurement among Metro Manila citites and municipalities (1).

These pollutants are known to be factors responsible for diminished life span due to onset of diseases: respiratory ailments, blood disorder, organic dysfunctions and cancer. Development of diseases could be alleviated if early warning signs such as occurrence of gene mutations are detected early enough, thus preventing the individual from further exposure to the mutagens.

The adapted hypoxanthine guanine phosphoribosyl transferase (HGPRT) mutation assay developed in our laboratory measures the degree of mutation on the HGPRT gene and

allows a rapid evaluation of the occurrence of harmful mutations in an individual exposed to radiation or mutagens within six months after exposure.

To address the problem on the effect of pollution on health and well-being of residents of Valenzuela, Metro Manila, a research contract with the Philippine Council for Health Research and Development was obtained starting from 15 May 1994 to 30 May 1995. The objectives of this project are as follows:

1. Apply the hypoxanthine guanine phosphoribosyl transferase (HGPRT) mutation assay to selected groups of Metro Manila residents exposed to environmental pollution;

2. Determine specific population groups in Metro Manila who are significantly affected by pollutants as evidenced by the increase in their peripheral blood lymphocytic HGPRT gene mutation index, and

3. Construct an environmental baseline HGPRT mutation data bank (EBMD) specific to the area in Metro Manila being sampled.

The study is undertaken by the project leader and her research assisstants at the Health Physics Research, section of the Atomic Research Division, Philippine Nuclear Research Institute in collaboration with the National Kidney Institute, Department of Health, where part of the research work is being done. Residents of Valenzuela, Metro Manila have been chosen as initial group donors for peripheral blood lymphocytes. Valenzuela has been identified by the Air Quality Monitoring Service (AQMS)/DENR-NCR as the metropolitan area having the highest atmospheric levels of total soluble particulates and nitrous oxide among the cities and towns comprising Metro Manila (1). The results of the study will ascertain if a correlation exists between increased incidence of HGPRT mutation among residents and an elevated level of environmental pollution in Valenzuela

METHODOLOGY

1. *Establishment of Linkages*

To identify the different areas as possible sampling sites, the Air Quality Monitoring Service of DENR-NCR which conducts air sampling analysis in the area was consulted.

The Committee on Environmental Protection of Valenzuela under the Chairmanship of Councilor Virginia Hernandez was presented with the research protocol to seek their help in finding possible blood donors in the municipality.

The Valenzuela Municipal Health Office was requested for the use of the clinic for blood extraction from donors by the municipal medical technologist.

Mr. Romy Dy, head of the Cellular Laboratory of the Immunology Department of the National Kidney Institute (NKI) has joined the project as collaborator/consultant. Part of the reseacrh work will be done at the NKI.

2. *Identification of Critical Areas in Valenzuela*

In consultation with a field epidemiologist, a sampling strategy was worked out and critical areas identified. These include Barangays Karuhatan and Balubaran which are within ten kilometer radius from the AQMS/DENR-NCR air sampler positioned in the municipal hall building.

Specific population groups classified in terms of age, smoking habit, occupation (indoor or outdoor), lifestyle and medical history were identified as possible blood donors. These data were accomplished in the prepared questionnaires printed in English and Pilipino.

3. *Peripheral Blood Collection*

Blood samples collected from donors using heparinized vacutainer syringes will be treated in accordance with the developed HGPRT assay (2) following the protocol enumerated below.

3.1 Lymphocyte isolation:

Mononuclear cells at the plasma/ficoll interphase is added to medium and centrifuged. Isolated cells are counted using hemocytometer after testing for viability with tryphan blue solution. Samples with less than 75% viable cells are discarded.

3.2 T-cell incubation:

Phytohemagglutinin (PHA) and 6-thioguanine (TG) are added to lymphocytes in solution. Cells are incubated in a CO_2 humidified chamber. Further incubation of cell solution in the presence of H-3 thymidine is done for 18 h at 37°C in a CO_2 incubator.

3.3 Radioactivity counts:

In vitro synthesis of DNA by lymphocytes in culture under the influence of PHA is terminated by placing the cell suspension on ice. Cells are precipitated with 10% trichloroacetic acid (TCA) (final concentration of 5%) in the cold and filtered through a Millipore filter system using a GF/C filter. Precipitates are washed with 10 ml volume of 10% TCA and dried with ethanol followed with acetone. Scintillation fluor (5 ml) is added to the dried filter and counted for radioactivity in the liquid scintillation counter (LSC). This represents the quantity (T). Control samples include cells cultured without PHA representing background cells and cells cultured without TG representing proliferating T-cells (C). All samples are analyzed in replicates.

3.4 DNA concentration:

An aliqout of TCA-treated cell solution is analyzed for DNA concentration. The acid insoluble fraction containing DNA and proteins are treated with ethanol, heated and centrifuged. The alcohol extract is acidified with perchloric acid, boiled for 30 min and centrifuged to separate the protein (3). The soluble fraction containing cellular DNA was measured at 260/280 nm using UV spectrophotometry for DNA concentration (4).

3.5 Specific activity:

Specific cellular activity is calculated based on the definition as CPM/cell count or CPM/DNA concentration.

RESULTS AND DISCUSSION

Table I represents a composite table of information on the medical and personal background and the corresponding mutation index values of blood donors residing in Barangays Karuhatan and Balubaran. The specific activities of H-3 labeled HGPRT based on cell counts at 0 h and 24 h were not very different from each other. This result allowed us to use with confidence either 0 h or 24 h cell count values in our calculations. The calculated mutation indices of samples based on DNA concentration showed values very close to mutation indices based on cell counts. From this result, it is possible to report mutation index values based on either of the two methods.

Table II shows the cumulative frequency distribution of mutation index values calculated from specific activity of H-3 labeled HGPRT based on either cell counts at 0 h and DNA concentration. Mutation index values lower than 0.4 represent mutations occuring spontaneously in cells. From the table, it is clear that there is a high number of samples with mutation index values greater than 0.5 indicating that about 30% of the total samples analyzed have mutation at the HGPRT gene.

It has been suggested that smoking affects mutation at the HGPRT gene (5). Our data on table III shows slightly higher values for mutation among smokers: however, the total number of samples used so far may be on the low side and definite conclusions cannot be drawn from such numbers. Table IV shows mutation at a particular age group. Values for both age categories seem not to differ from one another. Again, the total number of samples analyzed so far is on the low side level and therefore values may change with more samples.

We have tried to determine whether working outside and being exposed to environmental pollution for more than 5 h each day may affect the mutation indices of donors residing in Valenzuela. Table V is the mean average mutation indices at 5 or more hours of outdoor exposure to environmental pollutions. So far, the data show a slightly higher mutation rate among donors who are smokers with more than 5 hours outdoor exposure to pollutants per day than the corresponding class of non-smokers. The number of samples so far reported in the above categories are, however, still on the low side and await further confirmation.

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TABLE 1
HGPRT MUTATION INDEX

Sample	Age	Sex	M.I., 0 hr		No.of hrs outdoor	Occupation	M.I., [DNA]	
			nS	S			nS	S
160894 A	25	M		0.7560	8	welder		0.8000
B	29	M		1.2759	8	latero		1.1538
230894	27	M	0.0928		8	leg. ass.	0.1857	
270994 A	42	F	0.5106		2	housewife	0.5156	
B	34	M	1.1290		2	Eng'g. aide	1.0385	
181094	65	M	0.5339		3	util. worker	0.6853	
251094 A	41	M		0.3985	> 8	traff. enf.		1.1208
B	56	M		0.2130	8	traff. enf.		0.2538
081194	24	M		0.4424	4	off. worker		0.7194
151194 A	25	M	0.1128		8	traff. pol.	0.2689	
B	28	M		0.1285	8	traff. pol.		0.1892
221194 A	36	M		0.1035	8	sec. agent		0.0878
B	55	M	0.2657		8	traff. enf.	1.0000	
061294 A	44	F	0.0487		1	nurse	0.0600	
B	40	M		0.0911	2	janitor		0.1429
131294 A	21	M		0.0542	12	tricy. driver		0.0532
B	19	M		0.1370	12	tricy. driver		0.2026
040195 A	26	M		0.2148	8	elec. maint.		0.1107
B	24	M	0.2404		8	pintor	0.4176	
100195 A	39	M		0.3218	10	driver		0.2424
B	19	M	0.0661		12	-------	0.0868	
170195 A	44	F		0.1623	2	sanidad		0.1228
B	52	F	0.1637		2	midwife	0.1714	
240195 A	37	F		0.4162	8	plas. cutter		0.0957
B	34	F	0.2864		2	housewife	0.3456	
310195 A	48	F	0.3115		8	sec. agent	0.3814	
B	30	F	0.3238		1	housewife	0.2701	
070295 A	26	M		1.1317	10	pintor		1.3923
B	23	M		0.1088	24	taxi driver		0.3529
140295 A	20	M		2.1130	8-10	pahinante		1.3511
B	23	M		0.6512	12	latero		0.3185
220295 A	39	M		0.3333	12	fac. worker.		0.3216
B	27	M		0.3179	12	tricy. driver		0.3603
280295 A	25	M		0.7438	6	leg. staff assistant.		0.8433
B	28	M	0.9655		15	tricy. boy	0.8072	
070395 A	32	M		0.8601	4	operator		0.9048
B	32	M		1.2430	6	sewer		1.6024
140395 A	28	M		0.6478	10	laborer		0.5034
B	31	M	0.4476		14	fac. worker	0.3587	
200395 A	30	M		0.6145	10	driver		0.4300
B	38	F	0.7410		2	midwife	0.6667	
280395 A	39	F	0.4574		2	manamahi	0.5865	
B	44	F	0.5341		2	wallet maker	0.5038	

nS : non-smoker
S : smoker

Table II. Frequency Distribution of HGPRT Mutation for Exposure to Environmental Pollution

	Frequency Distribution of HGPRT Mutation					
	> 0.1	> 0.2	> 0.3	> 0.4	> 0.5	> 0.6
Cell Counts (0 Hr)	88.4%	69.8%	60.5%	46.5%	37.2%	27.9%
[DNA]	88.4%	74.4%	62.8%	46.5%	41.9%	32.6%

N=43

Table III. Average Mutation Indices (Mean ± Standard Deviation)

	Smoker N=25	Non smoker N=18
[DNA]	0.5470 ± 0.4600 Range = 0.0532-1.6024	0.4639 ± 0.2811 Range = 0.06-1.0385

Table IV. Average Mutation Indices at a Particular Age Group (Mean ± Standard Deviation)

	15-30 yrs old N=21	31 yrs old & above N=22
[DNA]	0.5151 ± 0.3959 Range = 0.0532-1.3923	0.5095 ± 0.3985 Range = 0.06-1.6024

Table V. Mean Average of Mutation Indices at <5 Hours and ≥5 Hours of Outdoor Exposure to Environmental Pollutants

	Mean Average Mutation Indices [DNA]	
	<5 h	**≥5 h**
Smoker	**0.4725 ± 0.3995 N = 4**	**0.5612 ± 0.4891 N = 21**
Non-smoker	**0.4800 ± 0.2859 N = 10**	**0.4383 ± 0.3111 N = 8**