

Collaboration between centres of the World Health Organization. Italy supports a Mexican University

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KEY WORDS

Italy-Mexico collaboration; epigenetic markers; vulnerable population

SUMMARY

Background: *Mexico is the third most populous country in America. A sizeable percentage of Mexicans live under unfavorable conditions such as malnutrition, marginalization and overcrowding. Environmental injustice includes: indigenous exposed to wood smoke, families living in mining zones, children working and living in garbage dumps and brick factories. Polycyclic aromatic hydrocarbons, pesticides, electronic waste and heavy metals exposure represent a health risk. These pollutants can induce biochemical lesions as DNA damage and epigenetic changes which could modify the gene expression profile of each individual. Objectives:* *The aim of this work is to create an Italy-Mexico collaboration (within the context of WHO collaborating centres) between the Center of Molecular and Genetic Epidemiology, University of Milan and the University of San Luis Potosí, will allow molecular approaches to develop early indicators of susceptibility to adult disease and cancer.*

RIASSUNTO

«Una collaborazione Italia-Messico in ambito dell'Organizzazione Mondiale della Sanità». *Il Messico è il terzo paese più popoloso d'America. Una percentuale considerevole di messicani vivono in condizioni sfavorevoli come la malnutrizione, l'emarginazione e sovraffollamento. L'ingiustizia ambientale comprende: indigeni esposti al fumo prodotto dal legno che brucia, famiglie che vivono in zone minerarie, bambini che vivono e lavorano nelle discariche e nelle fabbriche di laterizi. L'esposizione ad idrocarburi policiclici aromatici, pesticidi, rifiuti di apparecchiature elettriche ed elettroniche e metalli pesanti rappresentano un rischio per la salute. Questi inquinanti possono indurre lesioni biochimiche quali il danno al DNA e i cambiamenti epigenetici che potrebbero modificare il profilo di espressione genica di ciascun individuo. Una collaborazione Italia-Messico (nel contesto di centri collaboratori OMS) tra il Centro di Epidemiologia Molecolare e Genetica dell'Università degli Studi di Milano e l'Università di San Luis Potosí, consentirà un approccio molecolare mirato a proporre indicatori precoci di predisposizione alle malattie negli adulti e al cancro.*

INTRODUZIONE

According to the World Health Organization (WHO), a collaborating centre is an institution

designated by the Director-General to carry out activities in support of the Organization's programs. About 800 WHO collaborating centres around the world cooperate with WHO on several

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areas, including occupational health, nutrition, mental health, chronic diseases and health technologies. Currently, Mexico has seven collaborating centres, while Italy has twenty-three. One of the two Mexican centres outside Mexico City is located in San Luis Potosi. The focus of the Center at San Luis Potosi is Health Risk Assessment and Children's Environmental Health. This Center is part of the University of San Luis Potosi and has conducted several studies throughout the country. Relevant findings from recent research include: a screening for persistent organic pollutants and metals in Mexican children (aged 6-12 years) living in high-risk areas associated with mining, agriculture, oil fields, industry, and non-controlled waste disposal sites from communities located in nine Mexican states that showed high organochlorine insecticides, Polychlorinated Biphenyls and metals exposure. Although this is not representative of the Mexican population, a considerable group of children are exposed to chemicals and some at risk levels (19). A second study assessed exposures to arsenic and lead of children living near a copper-smelter in San Luis Potosi. Children aged 3-6 years had the highest mean blood lead levels and total urinary arsenic was higher in children aged 8-9 yr (7). Also, a health risk assessment in another mining area of Mexico found that children living in this area had higher lead blood concentrations (geometric mean of 13.8 microg/dL) and urinary arsenic levels (geometric mean of 52.1 microg/g creatinine). A positive correlation was found between exposure levels and DNA damage (13). Finally, exposure to mixtures of organic compounds and gases from biomass combustion for food cooking was determined in an indigenous community. Blood carboxyhemoglobin (% COHb), DNA damage in nucleated blood cells, and urinary 1-hydroxypyrene levels were measured. One month after an intervention program was applied -including the installation of ecological stoves and risk communication-, determinations were repeated. Levels of COHb were reduced to below 2.5% (mean level 1.0%) one month after the intervention. Moreover, when compared, DNA damage in people exposed before the intervention was significantly higher than when the program was in-

troduced and similar trend was observed with respect to urinary 1-hydroxypyrene levels (18).

Given these challenges, molecular epigenetic approaches have been considered to identify environmental impacts even at short exposure time as well as at low concentrations of different pollutants. The collaboration with The Center of Molecular and Genetic Epidemiology, University of Milan will provide a new perspective for assessing the environmental health impact from the use of epigenetic markers such as DNA methylation, histone modification and microRNA.

METHODS

The methodology used by The Center of Molecular and Genetic Epidemiology, University of Milan to determine epigenetic markers is briefly explained below.

DNA methylation measurement. Pyrosequencing is used for measuring both global DNA methylation and gene-specific promoter methylation changes via Alu and LINE-1 assays of bisulfite-treated DNA in peripheral blood lymphocytes (16, 17).

Modification of histone proteins in chromatin. Total histones are extracted from blood to determine histone H3K4 dimethylation (H3K4me2) and H3K9 acetylation (H3K9ac) by Enzyme-linked immunosorbent assay (8).

Micro ARN analysis. RNA isolation from the buffy coats to detect and quantify mature miRNAs is performed for subsequent reverse transcription and Real-time polymerase chain reaction (5).

DISCUSSION

In summary, three Mexican scenarios have been considered as major environmental problems: 1) Mining sites contaminated with heavy metals; 2) exposure to wood smoke and pesticides in tropical areas; 3) brick factories in suburban zones (figure 1a).

Children are particularly important because many of adult-onset diseases could be explained by fetal-stage exposures. Therefore, work during pregnancy and child labour represent relevant risk fac-

tors. According to WHO, environmental risk factors play a role in more than 80% of the diseases regularly reported. Also, children are particularly suitable for monitoring programs, as they are not directly exposed to occupational hazards; thus, children normally reflect trends of environmental exposure more accurately than do adults (14).

With the advent of molecular epidemiology, genetic and molecular markers can be used to monitor the impact of environmental toxicants on children's health. However, developing countries have limited technology and resources to conduct such studies. Therefore, it is essential to promote exchanges and collaborations with experienced centers in molecular epidemiology with robust technological support. The Center of Molecular and Genetic Epidemiology led by Dr. Andrea Baccarelli is part of the Department of Occupational and Environmental Health "Clinica L. Devoto", which is a WHO collaborating centre located in Milan, Italy (figure 1b). The Center's activity is focused on environmental impact assessment on human health through the identification of molecular and epigenetic factors. Epigenetics is particularly important because several toxicants have been shown to di-

srupt the epigenetic equilibrium (1, 2, 4). Epigenetics investigate changes in gene expression that are mitotically stable and do not depend on the underlying DNA sequence (i.e., genetics). Some epigenetic traits have even shown transgenerational inheritance in animal models. Because the epigenome is influenced by environmental factors throughout life, these changes can be induced since the fetal embryonic stage. Epigenetics mechanisms represent a form of cellular memory that can link early-life events in adult-onset disease. Dr. Baccarelli's group has shown biological and epigenetic perturbations induced by environmental factors under different exposure scenarios (table 1).

Two major epigenomic modifications are the methylation of DNA and the posttranslational modification of histone proteins in chromatin. Forty percent of human genes contain CpG-rich islands upstream from their transcriptional start site, and up to 70% to 80% of all CpG dinucleotides in the genome are methylated (3). When such widespread methylation occurs, it likely silences the gene by interfering with the access of transcription factors to the promoter region (9). The accumulation of methylation at the promoters of tumor sup-

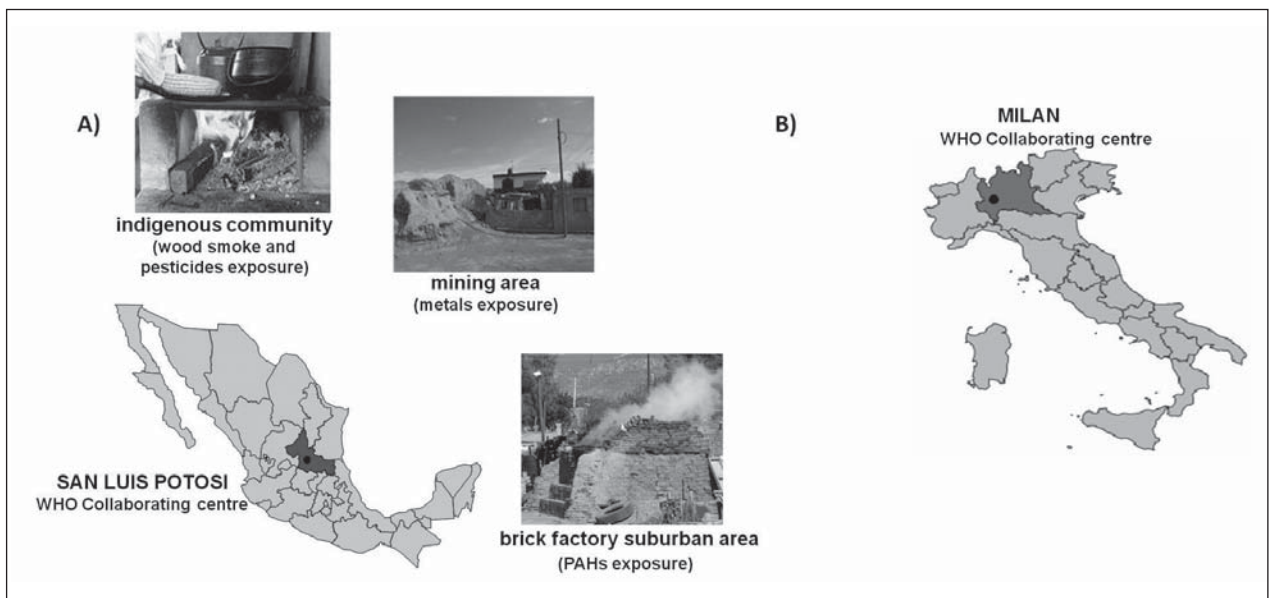


Figure 1 - Two collaborating centres of the World Health Organization. A) Environmental problems in Mexico: Three different exposure scenarios and the Center for Children Environmental Health. B) Center of Molecular and Genetic Epidemiology for the environmental impact assessment in Milan, Italy

Table 1 - Epigenetic and molecular markers of environmental exposure in different work areas

Scenario	Effect	Reference
Workers at an electric-furnace steel plant exposed to metal-rich particulate matter	Change in the expression of microRNAs in peripheral blood leukocytes	5
Workers exposed to polycyclic aromatic hydrocarbons	Shorter telomere length in peripheral blood lymphocytes	15
Traffic officers and indoor office workers exposed to traffic pollution	Leukocyte telomere shortening	11
Coke-oven workers exposed chronically to polycyclic aromatic hydrocarbons (PAHs)	Methylation states of gene-specific promoters (p53, p16, HIC1 and IL-6)	16
workers in an electric furnace steel plant exposed to PM < 10 microm	Significant demethylation of iNOS promoter	17
Elderly residents of metropolitan area exposed to traffic particles	Reduction of DNA methylation in heavily methylated sequences (LINE-1)	2
Gas station attendants exposed to benzene	Hypermethylation in p15 and hypomethylation in MAGE-1	6

pressor could predispose to develop cancer (10). An unfavorable factor that may affect the DNA methylation levels is malnutrition since nutritional factors affect the expression of specific genes by epigenetic modification, and these may be passed on to subsequent generations with potentially detrimental effects. For example, dietary deficiency in folate leads to genomic hypomethylation because this nutrient is an essential factor in the conversion of methionine to S-adenosylmethionine, the main methyl group donor in DNA methylation reactions (12).

Some of these molecular markers could be very useful to conduct molecular surveillance studies on vulnerable populations. Access to training and capacity building of human resources in molecular epidemiology are essential for scientific progress in developing countries. Currently, a Mexican institution, which promotes scientific and technological development (CONACYT), sponsors one post-doctoral position to train a Mexican scientist in the Center of Molecular and Genetic Epidemiology of Milan, Italy. We trust that this ongoing endeavor will be beneficial to Environmental Health both in

Mexico and Italy and represent a novel initiative leading to long lasting relations between the two WHO collaborating centres.

NO POTENTIAL CONFLICT OF INTEREST RELEVANT TO THIS ARTICLE WAS REPORTED

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