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***Mini-Review Article***

# MOLECULAR EPIDEMIOLOGY: A BETTER APPROACH FOR THE EARLY DETECTION OF PATHOPHYSIOLOGIC RESPONSE TO ENVIRONMENTAL TOXICANTS AND DISEASE

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*Our environment is becoming increasingly contaminated by a profusion of substances in the form of industrial and Municipal Waste, air and water pollutants; by heavy metals (such as lead) herbicides, pesticides, cosmetics and so on. The number of chemicals that affect man increases at alarming rates. These agents may be dangerous because they produce biochemical, genetic, structural or physiological lesions in a significant segment of the population. The importance of elucidating the nature and the mechanisms of physiological and toxicological reactions has been emphasized in the investigations of occupational and environmental diseases, such investigations have revealed that the clinical manifestations of intoxication may have their origin in injurious effects of subcellular or biochemical types. Slight to moderate derangements in metabolism may impair the functional activity of organs and lead to subclinical or overt clinical effects. These may elude detection or recognition of their health implications unless biomarkers, the functional components of molecular epidemiology are employed. Molecular epidemiology is an approach which aims to examine aetiology of disease in a more precise way by focusing on biomarkers of disease risk rather than relying on the actual occurrence of disease. Such studies can be carried out in a short time and with relatively small numbers of subjects compared with conventional epidemiology, which though currently more popular merely reveals association, and causal links often remain obscure. Detection of early biochemical lesions that are related to subsequent changes in structure and physiology would be useful as early indicators of environmental hazards that produce disease in humans, that is by employing molecular epidemiology. This will be greatly enhanced by newer tools, such as toxicogenomics and metabonomics.*

***Key words:*** *Molecular epidemiology, Environmental toxicant, Pollutants, Lead, Pathophysiologic response, , Toxicogenomics.*

## INTRODUCTION

As societies throughout the world are increasingly moving to greater levels of urbanization and industrial development, public concern is mounting over the state of the environment and much attention is now being paid to improving the environment for future generations. Probably the most disturbing aspect of the attendant pollution to urbanization and industrial development is the increasing presence of toxic chemicals in the natural environment. The large scale production and application of synthetic chemicals and their subsequent pollution of the environment is now a problem of serious concern in most industrialized countries and must be viewed as an extreme threat to the self-regulating capacity of the biosphere in which we all live.

The environment in the developing World including Nigeria as a result of progressive industrialization, which entails the increasing use of various chemicals, is also becoming increasingly contaminated by a profusion of substances in the form of industrial and municipal waste, air, water pollution by heavy metals, herbicides, pesticides, cosmetics food additives etc.

The number of chemicals that affects man increases at an alarming rate (Brodie, 1970). These agents may be dangerous because they produce biochemical or physiological lesions in a significant segment of the population.

The importance of elucidating the nature and the mechanisms of physiological and toxicological reactions has been emphasized in the investigations of occupational and environmental disease (De Bruin, 1971), such investigations have revealed that the clinical manifestations of intoxication may have their origin in injurious effects of subcellular

biochemical types. Slight to moderate derangement in metabolism may impair the functional activity of organs and lead to subclinical or distinct clinical effects. These may elude detection or recognition of their implications unless biomarkers, the functional constituents of molecular epidemiology are employed. Too often in the past such hazards have been defined only after outbrakes of human cases have occurred usually by techniques of conventional epidemiology.

The detection of early biochemical lesions that are related to subsequent changes in structure and physiology would be useful as early biomarkers of environmental hazards that produce disease in humans perhaps even more important in the long term is basic understanding of mechanisms by which environmental chemicals produce their effects which appear as the only rational basis for predicting the hazards associated with the mixture of chemicals currently in use globally.

**Lead Toxicity: A Prime Example of Environmental Pollution**

Since the age of metals began when man first learnt to extract them from ores and work them, exposures to industrial lead have vastly increased. Man was exposed to lead in Asia Minor 4500 years ago as a by – product of silver smelting (Schroeder, 1973) owing to its being an insidious and slow acting toxin, the use of lead by man continued for 4300 years without suspecting toxicity. Only since about two and a half centuries has he become aware of some of the extreme toxic effects of lead and learned to avoid them. In the past eight years more and more of these effects have been reported. Low level lead exposure that was previously thought innocuous is now also of great concern (Needleman and Allred, 1990).

The highest known exposures of human beings to lead before the age of petrol probably occurred in ancient Rome. Here amphorae stored syrups and wine, lead pipe carried water to the houses of the rich Romans, Soft water dissolves lead, lead cosmetics were used by the ladies. There is little doubt that lead poisoning was endemic among those who could afford such luxury. Infact (Gilfillan, 1965) believes that lead poisoning resulting in still births, spontaneous abortion (lead has been used as an abortificent) and sterility was responsible for the low birth rate of the upper classes of the Roman empire which led to the ultimate fall of the Roman Empire. It is not also known if the global environmental pollution accounts for the decrease in fertility and drop in sperm count that are currently being observed (Carlsen et al, 1992). The male reproductive system is known to be highly sensitive to some physical and chemical environmental exposures (Severer and Hassel, 1985). Simple biomarkers like urinary creatine may serve as early warning sign (Anetor et al, 2000).

There was little lead found in the bones of third century monks, but large amounts have been reported in those of the eleventh to the nineteenth century. This may even be greater in our own generation. Nigeria has one of the World's highest lead content in petrol )Thomas et al, 1999).

Africa's contribution to lead (Nigeria is one of the topmost rapidly developing and restructuring countries in Africa) to global lead pollution has increased from 5% in 1980 to 20% in 1996 (Nriagu et al, 1996). Additionally the blood lead level BLL in the children of many African countries have been reported to be excessive in some cities. Ninety per cent (90%) of children in Africa carry BLL above the current safe threshold (Nriagu, 1966).

Many reports have consistently indicated that our own environment (Nigerian) (Nriagu, 1996) is highly polluted. The increasing environmental pollution given by high BLL in the general population (Okoye, 1994, Adeniyi and Anetor, 1999, Anetor and Adeniyi, 2001) is largely attributable to the high lead content of our gasoline.

**Biomarkers in Environmental Toxicology**

In order to assess the health risks of exposure to potentially toxic chemicals biomarkers are essential. The use of and development of biomarkers has recently become of understandably major interest (Timbrel et al, 1994). With developments in analytical chemistry and biochemistry, methods have become available to trace the fate of environmental chemicals in the human body so as to assess the chemical exposure status of an individual and the risk of disease development by biomarker determination. Biomarkers can be used for preventive purposes and for risk assessment. Some sensitive techniques have been developed to detect DNA damage in human or animal cells. These are being used to assess the impact of pollutant exposure or genotoxic changes in both the general population and industrial workers in some countries in Europe and Latin

America (Restrepo et al, 2000). The early detection of occupational diseases is of prime importance as initial changes are often reversible.

**Molecular Epidemiology in Environmental Pollution**

Classical epidemiology is the scientific study of disease distribution and determinants. Epidemiology, however, merely reveals associations and causal links often remain obscure. Molecular epidemiology appears superior to traditional epidemiology in the early detection of early biochemical lesions that lead to subsequent changes in structure and physiology. Molecular epidemiology may be considered to be the epidemiological correlate of molecular toxicology which rationalizes on molecular basis the toxicological responses of the organism to environmental, industrial and domestic chemicals thus providing a firm basis for evaluation of risk to man. Molecular epidemiology is an approach which aims to examine aetiology of disease in a more precise way by focusing on biomarkers of disease risk, rather than relying on the actual occurrence of disease. Such studies can be carried out in a short time and with relatively small numbers of subjects compared with conventional epidemiology, which though popular however, merely reveals association and causal links often remain uncertain. Table 1 shows some of the benefits of Molecular Epidemiology.

**Molecular Epidemiology, Toxico-genomics and Metabonomics**

Toxicology is the science of the adverse effects of chemicals, drugs, environmental agents and stressors. Genomics, defines the structure, sequence (code) and function of the entire DNA complement of organisms. The interface of these diverse disciplines is called toxicogenomics and is based upon the application of genomics technologies to define globally the changes in gene expression (both RNA and proteins as a consequence of exposure to environmental toxicants (Tennant and Selkirk, 2002). DNA microarray technology enables the simultaneous measurement of transcription of thousands of genes using micro-chips containing thousands of probes of complementary DNA (cDNA) immobilized in a predetermined array. The ultimate application of this technology to environmental toxicology or indeed general toxicology holds great promise for molecular epidemiology, though currently faces several formidable problems that need to be surmounted. Microarray assay approaches have been proposed to investigate the mechanism of action of endocrine disruptors and as a potential screening method for synthetic and natural endocrine disruptors (Tennant and Selkirk, 2002). The situation of endocrine disruptors in Nigeria is currently unknown although this is predicted to be very high (Anetor, 2002). This is a current concern of the scientific community, especially SCOPE (Scientific Committee on Problem of the Environment) and IUPAC (International Union of Pure and Applied Chemistry). Molecular epidemiology will play a great role in this respect in investigating this problem in this environment.

The problem of identifying environmental factors involved in the induction and evolution of human disease, and in conducting safety and risk assessments of drugs and chemicals, have long been formidable issues. Three major component for predicting potential human health risk are firstly, the diverse structure and properties of a huge number (thousands) of chemicals and other environmental stressors; Secondly, the time and dose parameters that underlie the relationship between exposure and disease and thirdly, genetic diversity of surrogates to assess adverse chemical effects. The techniques evolving from the successful genomics efforts are providing new tools with which to address these intractable problems of environmental health and toxicology.

The simultaneous analysis of expression of thousands of genes as end points using cDNA chips or microarrays should allow toxicologists a new comprehension of toxicological issues. Toxicogenomics, the combined field of toxicology and genomics thus has become a focus for the research community and regulatory authorities as a new approach to understanding of the Mechanisms invoived. It can provide us with very useful data relevant.to difficult areas such as dose-response relationships, species-to-species extrapolations and exposure assessment that can not be resolved by traditional toxicological techniques (Shirai and Asamoto, 2002). Application, of toxicogenomics technology to environmental toxicology issues can be expected to overcome the limitations of conventional methods. A great possibility is that toxicogenomics will facilitate differentiation of gene responses specific to organ system activity from those associated with non-specific general response.

It can be envisaged that efforts with specific organs like the mammary glands, endometrium, prostate gland and the thyroid depending upon hormonal activities would be rewarding. Confidence in results might be raised by combining toxicogenomics thus molecular epidemiology with traditional toxicological and toxicolpathological findings. Though current efforts are focused on Endocrine disruption especially the reproductive pathway (Damstra et al, 2002), it holds great promise for toxicology especially environmental toxicology.

Closely rated to toxicogenomics is the application of products and by-products of metabolism. Metabolites are the products of the many intricate biosynthesis and catabolism pathways that exist in human and other living systems. Historically, measurement of metabolites in human biofluids has been employed for the diagnosis of a number of inherited disorders and for assessing exposure to certain xenobiotics. Traditional analysis approaches have focused on one or a few metabolites.

In recent times, advances in analytical separation and detection methods, coupled with developments in bioinformatics, have made it possible to measure and interprete complextime-related metabolite profiles that are present in biological fluids. The terms metabonomics and metabolomics have been coined to describe metabolic profiling, although the precise nomenclature including potential differences between these terms, is still evolving. The application of metabonomics to study potential environmental contributions to disease was recently examined at the Division of Extramural Research and Training Science Planning retreat held 27-28 November 2001 in South Pines, North Carolina (Lawler, 2002). The session highlighted the opportunities and challenges provided by metabolic profiling which will be used as guidelines of future attempts by the National Institute of Environmental Health Sciences (NIEHS) to promote and support the application of molecular and related principles to environmental health sciences and their full integration to future genomic and proteomic initiatives. Metabonomics provides an integrated detector of both primary and secondary disturbances that point to a pathophysiologic process, genetic modification or xenobiotic exposure.

Abundant opportunities exist for the application of metabonomics to the field of environmental health sciences or toxicology particularly in the area of biomarkers of exposure and disease.

## Cancer and Environmental Toxicity

Although cancer is perhaps the most feared and best known of the chronic pathologic effects of environmental chemicals, it should be recognized that environmental agents may cause other forms of chronic illness including birth defects, reproductive impairments on behavioural abnormalities.

Environmental factors have however, been estimated to cause more than 85% of human cancers, but in such cases the term environmental is all inclusive and refers essentially to all causative agents other than genetic factors (Holbrook, 1990). Environmental factors may infact be responsible for 25% of human cancer, but such environmental chemicals include those manufactured, those resulting from the industrial or work environment, natural plant or fungus produced toxins e.g. aflatoxin and inorganic salts either metallic or nitrate/nitrite.

**Table I**
Some Specific Derivable Benefits from Molecular Epidemiology

- Serve as chemical and biochemical indicators of toxic exposure
- Biomarkers of damage to DNA
- Signs of early organ dysfunction
- Indicators of inherited hyper susceptibility
- Detection of individual sensitivity to chemicals
- Analytical and diagnostic validity
- Applications in biological monitoring and risk assessment
- Implications for preventive strategies
- Species-to-species extrapolation

In the case of cancer, measures of DNA damage and mutation are appropriate biomarkers. These are recognized as early events in the process of carcinogenesis. It is indeed possible that the current explosion of cases of cancer in the developed countries which is gradually creeping into the developing countries is probably due to dearth of methods of molecular epidemiology to pick the process of carcinogenesis at the initiation stage before it progresses to the phase of promotion.

## The Metabolic Fate of Environmental Chemical Transformation

The key enzymes involved in this process are the cytochrome p-450, a collective term for a

group of haemoproteins that bind foreign chemicals (Xenobiotics) and transform their oxidation by inserting one molecule of oxygen ($O_2$) oxygenation. Such hydroxylation reactions convert many water insolubic foreign compounds to more polar derivatives which are then more easily excreted. Often associated with conversion to polarbolites is loss of pharmacologic or toxicologic effects of drugs or other xenobiotics. These may serve as biomarkers of exposure. Biomarkers of exposure have been the most actively studied especially in the use of biomarkers of exposure to mutagenic and carcinogenic chemicals (Anonymous, 1992). In general terms, markers of exposure rely on measurement in body fluids or tissues of either the substance in question or its metabolites or a product of a reaction with a biological molecule.

Biomarkers of exposure can be divided into markers of internal dose and markers of effective dose. The former is an indicator of the occurrence and extent of exposure of the subject, where as the latter is an indicator of the extent of exposure of what is regarded to be the target molecule, structure or cell, thus amenable to molecular epidemiology.

Biomarkers of internal dose indicate that exposure to a particular compound has taken place by measuring the compound or its metabolites in body fluids. Although human exposure to a particular chemical may be estimated from biomonitoring studies using workplace monitoring for example or preferably personal monitors, there is individual variability in absorption, distribution and excretion. Consequently, it is preferable to measure the amount of compound or its metabolites in a tissue or fluid from a subject in order to estimate the actual exposure rather than the expected exposure.

**Biomarkers of Oxidative Damage**

Oxidative stress is a general term for a physiological or pathophysiological situation in which oxidative processes exceed the antioxidant defences of an organism. This principle has been utilized as selective and general biomarker of oxidative damage to DNA. The production of free radicals has been shown to be greatly increased to noxious environmental factors such as UV light, cigarette smoke and environmental pollution. Super oxidedismutase (SOD) is an enzyme used extensively as a biochemical indicator of pathological states associated with oxidative stress (Autor, 1982) because of the protective role it plays against deleterious effects triggered by superoxide anion in turn arising from environmentally induced free radicals (Kehrer, 1993).

**Molecular Epidemiology for an Environmentally Sustainable Future**

The current education of most professionals involved in the management of environmentally induced disorders is unquestionably incomplete. Cortese (1992) has suggested that scientists are needed to understand the natural World, the effects of human activity on the environment, the fate and transport of pollutants in the environment, and the efficacy of environmental improvement strategies. According to Cortese (1992) Health specialists on the otherhand, should help understand the effects of environmental pollution on human health and advise policy makers, patients, and the public on strategies to reduce health hazards. This can better be achieved by early detection of pathophysiologic changes employing molecular epidemiology. The multifactorial nature of toxic responses to environmental chemicals necessitates the use of early biomarkers of effects as well as biomarkers of exposure and susceptibility. It is essential that all of us understand the importance of the environment to our existence and quality of life and that we have the knowledge, tools, and sense of responsibility to discharge our duties to society and thus ensuring a sustainable future.

**Cautionary Note on the Use of Molecular Epidemiology**

There is no doubt now that molecular epidemiology is sufficiently sensitive and the preferred approach for the early detection of pathophysiologic response to environmental toxicants and disease. It is however, worthy of note that just because we can detect the presence of a chemical or measure a biochemical effect does not mean that this represents a hazard and therefore that the individual is at risk. Some biomarkers may be irrelevant to toxicity or too sensitive; a good example of which is inhibition of the activity of the enzyme of the haemopoietic system, d-aminolalvulinic acid dehydratase (ALA-D).

Part of this dilemma was about a decade ago revealed by the observation of some investigators that it is becoming more difficult to distinguish between measured alterations that are adaptive and reversible and those that are pathological and irreversible (Timbrell et al,

1992). Therefore, different biomarkers need to be used in conjunction where possible and appropriate. This is well illustrated by lead exposure which has been well characterized in humans.

## Conclusion

The nature of toxic substances that give rise to chronic poisoning varies ranging from elements, particularly metals through complex organic and inorganic compounds. These substances may be encountered as drugs, pesticides, industrial chemicals and pollutants. Generally they constitute a spectrum of substances in a variety of states in a multiplicity of matrices at extremely low concentrations. Thus their ability to bring about pathophysiological changes is not immediately evident. They constitute enormous challenges to both the analytical and clinical toxicologist in search of biomarkers that will subsequently be employed in molecular epidemiology. This ensures the early detection of biochemical lesions that are related to subsequent changes in structure and physiology. Thus useful as early indicators of environmental hazards that produce disease in humans.

This benefit will be greatly enhanced by the newer technologies of toxicogenomics and metabonomics.

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