THERAPEUTIC APPLICATIONS OF BIOMARKERS IN HEALTH ASSESSMENT AND TOXICITY

S. K. Singh¹, N. Kumar², A. Panicker³* and B. Meshram⁴

¹Assistant Professor/ Scientist, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science & A.H., Rewa
²Associate Professor/ Senior Scientist, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science & A.H., Rewa
³Teaching Associate, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science & A.H., Rewa
⁴Associate Professor, Department of Veterinary Anatomy and Histology, Khalsa College of Veterinary & Animal Sciences, Amritsar (Punjab)

ABSTRACT

The term "biomarker" is used in a broad sense to include almost any measurement reflecting an interaction between a biological system and an environmental agent, which may be chemical, physical or biological. Biomarkers are substances found in abnormal amount in body fluids or tissue, and classified as either a metabolite or molecular entity. It is a measurable characteristic in a biological system which changes due to disease, exposure to chemicals and other toxicants. Biomarkers may be used to assess the exposure and effects of chemicals and susceptibility of individuals. Biomarkers may be applied to elucidate cause-effect and dose-effect relationships in health risk assessment and in clinical diagnosis. Tumor markers such as surface antigens, cytoplasmic proteins, enzymes can be measured in serum or plasma. As such the tumor markers support the diagnosis of cancers, besides being useful for monitoring the response to therapy and for the early detection of recurrence. Discovery of new disease biomarkers and ability to measure them rapidly will revolutionize disease diagnosis.

Keywords: Biomarkers, toxicity, health assessment, clinical diagnosis, risk assessment.

INTRODUCTION

Successful treatment of diseases depends on early detection and appropriate therapy. Apart from history, clinical findings and physical status regarding disease condition, there are certain laboratory parameters in blood, urine, milk, cerebrospinal fluid, synovial fluid and tissues of various body organs which may be of diagnostic value in confirming cause and changes associated with that particular disease condition. Such characteristic
laboratory parameters and their level in respective condition may be used as biomarkers. Monitoring of metabolites levels to diagnose and predict disease has been around for some time, such as veterinary clinician routinely employs hematological and biochemical tests to diagnose problems such as vitamin B\textsubscript{12} and folate to diagnose anemia or neuropathy; and creatinine to diagnose problems of the kidney. Abnormally high or low levels of these chemical metabolite warrant medicinal intervention and can result in successful treatment.

Advances in research have also lead scientists to identify the presence of certain disease status by monitoring expression levels of DNA, RNA, proteins and chemical metabolites found in body fluids and tissue, the entities called biomarkers\textsuperscript{1}. The term "biomarker" is used in a broad sense to include almost any measurement reflecting an interaction between a biological system and an environmental agent, which may be chemical, physical or biological (fig.1). Biomarkers are substances found in abnormal amount in body fluids or tissue, and classified as either a metabolite or molecular entity. It is a measurable characteristic in a biological system which changes due to disease, exposure to chemicals or other factors\textsuperscript{2}.

Figure 1
Measurement of biomarkers
1. BIOMARKERS OF EXPOSURE
These include an exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism. The exposure assessment component of the risk assessment process is an attempt to provide qualitative and quantitative estimates of human exposure through the use of measurements and models. In this context, measurements may be made up of chemical concentrations in food, water and air, selected environmental concentrations like occupational or residential settings as well as measures of the actual exposure experienced by the individual or population. Adverse or toxic effects in a biological system are not produced by chemical agents unless that agent or its biotransformation products reach appropriate sites in the body at a concentration and for a length of time sufficient to produce the toxic manifestation. Thus, to characterize the potential hazard or toxicity of a specific chemical agent in an individual, it is necessary to identify not only the type of effect and the dose required to produce the effect but also information about the duration and frequency of exposure to the agent, and the susceptibility of the exposed individual.³

Methods for assessing exposure to a chemical fall into two categories:
1. Measurement of levels of chemical agents and their metabolites and/or derivatives in cells, tissue, body fluids or excreta.
2. Measurement of biological responses such as cytogenetic and reversible physiological changes in the exposed individuals.

In evaluating exposure, distinction should be made between the external dose, defined as the amount of a chemical agent in environmental contact with the organism, as determined by personal or area monitoring, and the internal dose, which is the total amount of a chemical agent absorbed by the organism over a period of time. Biomarkers of exposure will reflect the distribution of the chemical or its metabolite throughout the organism. Theoretically, this distribution can be tracked through various biological levels
(e.g., tissue, cell, etc.) to the ultimate target. The concept of biomarkers of exposure is mentioned below (figure-2).

![Figure-2: The pathway from exposure to overt clinical effect](image)

2. BIOMARKERS OF EFFECT

A measurable biochemical, physiological, behavioral or other alteration within an organism that, depending upon the magnitude, can be associated with an established or possible health impairment or disease. Biomarkers of effect may be used directly in hazard identification and dose-response assessment components of the risk assessment process. In hazard identification, biomarkers may facilitate screening and/or identification of a toxic agent and characterization of the associated toxicity. Biomarkers that are implicated in toxic mechanism(s) are preferred for quantitative dose-response assessments when extrapolating from existing data to an individual situation of concern (e.g., from high to low dose or from test species to humans).

There are wide inter-individual variations in the response to equivalent doses of chemicals. While the outcome of a chemical response in an individual may be predicted more accurately from biomarkers of effect(s), such biomarkers may not be specific for a single causative agent. Many biomarkers of effect are used in everyday practice to assist
in clinical diagnosis; however for preventive purposes an ideal biomarker of effect is one that measures reversible change. Nevertheless, certain biomarkers of nonreversible effects may still be very useful in epidemiological studies or provide the opportunity for early clinical intervention.

3. BIOMARKERS OF SUSCEPTIBILITY

It is an indicator of an inherent or acquired ability of an organism to respond to the challenge of exposure to a specific xenobiotic substance. Biomarkers of susceptibility focus on the genetic predisposition of an individual as it affects susceptibility to chemical materials. There are a number of external factors, such as age, diet and health status, that can also influence the susceptibility of an individual exposed to chemicals. Some discussion will be directed towards the effects of previous exposure on subsequent susceptibility, such as sensitization and enzyme induction/inhibition by previous exposure. Although individuals may experience similar environmental exposures, genetic differences in metabolism may produce markedly different doses at the target site and thus a different level of response is achieved. Even when target doses are similar, markedly different responses may be noted in individuals due to varying degrees of inherent biological responsiveness. Biomarkers of susceptibility may reflect the acquired or genetic factors that influence the response to exposure. These are pre-existing factors and are independent of the exposure. They are predominantly genetic in origin, although disease, physiological changes, medication and exposure to other environmental agents may also alter individual susceptibility. Biomarkers of susceptibility identify those individuals in a population who have an acquired or genetic difference in susceptibility to the effects of chemical exposure.

Biomarkers of susceptibility indicate the factors which may increase or decrease an individual's risk of developing a toxic response following exposure to an environmental agent. Polymorphism is present for some metabolic activation/deactivation enzymes, including cytochrome P-450 isozymes and at least one form of glutathione transferase.
The enzyme activity controlling the activation or detoxification of xenobiotics leads to differences in susceptibility by increasing or decreasing the biologically effective dose of the environmental agent.

Biomarker Concept and Exposure – Disease Continuum

Figure-3: Biomarkers pathway in relation with outcome of a disease

BIOMARKERS AND THE RISK ASSESSMENT PROCESS

In the assessment of risk, biomarkers may be used in hazard identification, exposure assessment and to associate a response with the probability of a disease outcome. By examining the interactions between host and chemical exposure, and comparable data for experimental studies of mammalian species, criteria for the selection of biomarkers indicative of exposure, effects, susceptibility and toxic response(s) to chemicals may be established.

Identification of practicable biomarkers associated with different toxic end-points or outcomes require interdisciplinary cooperation and research, and this is evident in relation to carcinogenesis, neurotoxicity, pulmonary toxicity, immunotoxicity and reproduction. The process for assessment of health risks associated with exposure to chemicals is multifaceted the following major components:

(A) Hazard Identification: To confirm that the chemical is capable, subject to appropriate circumstances, of causing an adverse effect.
(B) **Dose-response assessment**: To establish the quantitative relationship between dose and effect.

(C) **Exposure assessment**: To identify and define the exposures that occur, or are anticipated to occur, in populations.

Risk characterization is the synthesis of the qualitative and quantitative information that describes the estimated risk to human or animal health from the anticipated environmental exposure. Hazard identification and dose-response assessment make use of all available data for human and animal.

**APPLICATIONS OF BIOMARKERS**

Biomarkers may be used to assess the exposure (absorbed amount or internal dose) and effect(s) of chemicals and susceptibility of individuals, and they may be applied whether exposure has been from dietary, environmental or occupational sources. Biomarkers may be used to elucidate cause-effect and dose-effect relationships in health risk assessment, in clinical diagnosis and for monitoring purposes. Biomarkers of exposure can be used to confirm and assess the exposure of individuals or populations to a particular substance, providing a link between external exposures and internal dosimetry. Biomarkers of effect can be used to document either preclinical alterations or adverse health effects elicited by external exposure and absorption of a chemical. Thus the linkage of biomarkers between...
exposure and effect contributes to the definition of dose-response relationships. Biomarkers of susceptibility help to elucidate the degree of the response in individuals.

(A) BIOMARKERS IN HEALTH ASSESSMENT
Measurements carried out for many years within the context of "biological monitoring" have been used to assess worker exposure and, in clinical settings, to evaluate the administration of therapeutic agents. These measurements, or biomarkers, provide the critical link between chemical exposure, internal dose and health impairment, and are of value in assessment of risk. However, there is a need to identify and validate for each organ system those characteristic parameter(s) that are indicative of induced dysfunction, clinical toxicity or pathological change, as well as to establish the specificity and sensitivity of each biomarker and its method of measurement.

(B) BIOMARKERS FOR CLINICAL DIAGNOSIS
Biomarkers may be used to:
1. Confirm diagnosis of acute or chronic poisoning
2. Assess the effectiveness of treatment
3. Evaluate the prognosis of individual cases
For this purpose, a well-established relationship between biomarker (s) and outcome must be available. Assessment of exposure in short-term or long-term exposure situations can be evaluated on a more meaningful basis where previous exposure has been documented by consecutive measurements over a period of time. Although this may not be possible in the circumstances of a major chemical release however biomarkers of effect may still find useful application to assess clinical diagnosis.

(C) BIOMARKERS FOR MONITORING PURPOSES
Biomarkers may be used to confirm the exposure of individuals in a population to a particular substance, such as an organic solvent in exhaled breath, the cadmium burden of the kidney, lead in bone, or the fatty tissue storage of chlorinated hydrocarbons. Quantitative measurements may facilitate the determination of dose-response
relationships. Biomarkers are used for screening and for monitoring and may be determined and applied on an individual basis or may be related to a population group. Population groups "at risk" may be identified by deviations from normal mean values for biomarkers of exposure or effects. Some public and occupational health surveillance programmes include the use of biomarkers for screening and monitoring purposes.

**NEPHROTOXICITY BIOMARKER**

Several different types of measures have been tested and used as biomarkers of renal damage and have been classified as functional markers (e.g., serum creatinine and β2-microglobulin), urinary proteins of low or high molecular weight (e.g., albumin, transferrin, retinol-binding globulin, rheumatoid factor, immunoglobulin G), cytotoxicity markers (tubular antigens, e.g., BB50, BBA, HF5), enzymes (e.g., N-acetylglucosaminidase, β-galactosidase) in urine, and biochemical markers (eicosanoids, e.g., 6-keto PGF2α, PGE2, PGF2α and TXB2, fibronectin, kallikrein activity, sialic acid and glycosaminoglycans in urine, and red blood cell negative charges) 8.

**TABLE-1: SOME NEPHROTOXICITY MARKERS**

<table>
<thead>
<tr>
<th>ANALYTE</th>
<th>FUNCTION</th>
<th>DAMAGED REGION</th>
</tr>
</thead>
<tbody>
<tr>
<td>β2m</td>
<td>Small cell surface protein shed into the blood and normally reabsorbed by the proximal tubules of the kidney. High β2m levels result from lack of effect reassertion due to renal failure</td>
<td>Proximal tubule, glomerulus</td>
</tr>
<tr>
<td>KIM-1</td>
<td>Membrane protein expressed at elevated levels after injury of proximal tubule epithelial cells due to ischemic renal damage</td>
<td>Proximal tubule</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>Regulates extracellular matrix synthesis and degradation and, along with matrix metalloproteinases, is essential for tumor growth and health</td>
<td>Proximal tubule, distal tubule</td>
</tr>
<tr>
<td>Clusterin (apolipoprotein J)</td>
<td>Conserved protein induced during tissue injury or remodeling</td>
<td>Proximal tubule, distal tubule</td>
</tr>
</tbody>
</table>
Cystatin C | Extracellular inhibitor of cysteine proteases normally expressed in vascular wall smooth muscle cells | Glomerulus
---|---|---
Albumin | Most abundant plasma protein essential for maintaining the osmotic pressure; acts also as a plasma carrier by non specific binding of hydrophobic macromolecules | Glomerulus

| Total protein | Multiple proteins of serum and kidney | Nonspecific

β2m; β2-microglobulin; KIM-1 Kidney injury molecule 1; TIMP -1; tissue inhibitor of matrix metalloproteinase – 1.9

**LIVER TOXICITY BIOMARKERS**

Effects of chemicals on the liver have been estimated traditionally by measuring the activities of liver such as aminotransferase (most often aspartate or alanine aminotransferase) in the serum, where they are found when liver cells have been damaged and have leaked their contents. Many other enzymes have also been analysed.
for this purpose which include 5-nucleotidase, alcohol dehydrogenase, lactate dehydrogenase, isocitrate dehydrogenase, leucine aminopeptidase, glutathione S-transferase, ornithine carbamoyl transferase). Since tissues other than liver also contain these enzymes, their activities may be elevated in serum not only after liver damage but also when non-hepatic tissues have been damaged. To overcome this lack of specificity, analysis of specific isoenzymes has been used. Serum activities of enzymes such as alkaline phosphatase and gamma-glutamyl transpeptidase may be used as biomarkers of hepatic damage mainly involving biliary excretion. Several liver function tests can also be used as biomarkers of effects; these include the concentrations of serum proteins synthesized in the liver, e.g., albumin and clotting factors, or serum concentrations of bile acids, also synthesized in the liver, as well as tests for the hepatic excretory function such as bromsulphthalein half-time. These parameters lack specificity since hepatic viral infections, alcohol and drug use affects these enzymes. Indirect measures of chemically induced change(s) in the cytochrome P-450 enzyme system using provocation tests have been proposed as sensitive indicators. However, the relationship to liver damage and disease is not established and the requirement for the administration of a drug restricts the use of such tests.

Hepatotoxicity is caused by a number of chemicals that are metabolized by the cytochrome P-450-dependent mixed-function oxidase system to reactive intermediates. For example, carbon tetrachloride has been studied extensively; it is metabolized to a reactive intermediate which initially depletes intracellular glutathione to a level that is no longer protective when the metabolite reacts with critical macromolecules leading to cell death and hepatoxicity. In this example, “biomarkers of effect” could include glutathione levels, lipid peroxidation or the number of necrotic cells.

HAEMATOLOGICAL BIOMARKERS

Inhibition of the enzymes in the haem synthesis pathway (e.g., ferrochelatase, levulinate dehydratase) has been used as a marker of effect of exposure to lead. This effect is
reflected also in the levels of free erythrocyte protoporphyrin (FEP) and delta-aminolevulinate in the urine. Elevated levels of urinary delta-aminolevulinate are observed at higher lead exposures than changes in FEP such as while basophilic stippling of erythrocytes is an even less sensitive biomarker for the effects of lead. However, the effects on haem synthesis are not specific to lead as a causative agent; iron deficiency has a similar effect on FEP. The relationship of these effect biomarkers to toxicity requires further elucidation.

Routine leucocyte, erythrocyte and thrombocyte counts have been used in the surveillance of patients treated with cytostatic drugs and in the monitoring of benzene-exposed workers. The predictive power in relation to benzene-induced aplastic anaemia or leukaemia is limited. Ferrokinetic measurements, such as plasma iron disappearance half-time, erythrocyte utilization of iron, plasma iron transport rate, or erythrocyte iron turnover rate, have been suggested as biomarkers of myelotoxicity.

BIOMARKERS OF IMMUNOTOXICITY

The immune system protects the organism against infectious microorganisms and the growth of at least some neoplasms. Reactions of the immune system are influenced by genetic factors, age, nutrition, and life-style and health status. Xenobiotics may stimulate or suppress the immune system. After initial sensitization, even a minimal new exposure may lead to an anaphylactic reaction. The immune system may be more sensitive to chemical challenge than any other body system. Hypersensitivity reactions following inhalation exposure include asthma, rhinitis, pneumonitis and granulomatous pulmonary reactions. Hypersensitive dermal reactions induced by chemicals include a wide variety of acute, subchronic and chronic changes. Patch testing has been used traditionally as a biomarker for identification of the causative agent of an allergic skin reaction. However, the possibility of inducing hypersensitivity by patch testing has been well documented and should not be overlooked.
Elevated levels of specific antibodies, usually of the IgE type, may indicate existing sensitization. However, not all individuals with elevated levels are symptomatic and not all symptomatic individuals exhibit elevated IgE levels. Suppression of the immune system increases susceptibility to infections and neoplasia. Changes in the relative abundance of different lymphocyte subpopulations (suppressor and helper T-cells) have been used as biomarkers for the immune suppression. Individuals with asbestos-induced pleural or pulmonary changes, or asbestos-induced cancer, as well as those heavily exposed to asbestos but without apparent disease, have been reported to exhibit an altered immunological status (e.g., reductions in T-lymphocyte subsets).

BIOMARKERS OF PULMONARY TOXICITY

The most frequently used markers measure gross effects on pulmonary function are peak expiratory flow, forced expiratory volume and transfer factors. These measures tend to be nonspecific with respect to the causative agent and may overlook effects specific to a certain cell type. Peak expiratory flow measurements can be performed by the exposed individuals themselves at the workplace, at home or elsewhere, and they provide information on the underlying causes of air-way obstruction, allowing a closer association between exposure, atmosphere and response. Air-way hyperactivity can be assessed by challenge tests using inhalation exposure. Although such tests may assist in identifying the factors causing hypersensitive pulmonary reactions, there is a clear risk of acute reactions, and testing should be carried out by qualified personnel in carefully controlled environments.

Recently analysis of bronchoalveolar lavage fluid (BALF) has been used to detect lung injury or to follow the progress of pulmonary disease or the efficacy of therapeutic treatment. The use of cellular elements as markers of pulmonary disease state has been emphasized in BALF analysis. Total cell counts and differential counts, including use of monoclonal antibody staining to distinguish T-cell subtypes, are used to detect alveolitis and to aid in the diagnosis of interstitial lung disease. High percentages of lymphocytes
are indicators of granulomatous processes, such as sarcoidosis, or hypersensitivity pneumonitis. High percentages of neutrophils with some eosinophils indicate possible idiopathic pulmonary fibrosis. Other extracellular components, such as cytokines and other mediators of inflammation have been used on an experimental basis to answer specific research questions.

Nasal lavage fluid (NLF) also provides markers of response to inhaled toxins. NLF analysis to document the influx of neutrophils into the nasal cavity in humans in response to inhaled ozone. Biomarkers in blood related to lung injury have not been validated. However, it was reported that serum aminoterminal propeptide of type III procollagen (PIIINP) may become useful as an early marker for developing fibrosis. A dose-dependant increase in serum PIIINP was found in individuals exposed to low or high levels of asbestos. Finally, urinary levels of amino acids associated with the connective tissue of the lung (hydroxyproline, hydroxylysine, desmosine and isodesmosine) have been used as markers of lung injury.

BIOMARKERS OF REPRODUCTIVE AND PRENATAL TOXICITY

Markers associated with an adverse outcome in reproduction may reflect toxic effects in the male or female or be associated with development during the embryonic, fetal, perinatal or neonatal period. Biomarkers for the male reproductive system may include physiological indicators of impaired testicular function, or sperm number or characteristics (including cytogenetics). Measures of hormonal status such as FSH, LH and testosterone can also be readily obtained from blood and, in the case of testosterone, from urine and saliva. The clearer picture of hormonal status can be obtained by administering GnRH or LH and examining the hormone response to these challenges. Biomarkers for the male reproductive system are rather easily accessible and some even reasonably well validated however such markers are less well developed for the female reproductive system.
Biomarkers indicative of developmental toxicity should also be considered. These biomarkers include measurements of detrimental effects produced by chemical or other exposures during embryonic or fetal stages of development. Irreversible lesions can be embryolethal or result in functional anomalies in the offspring. Examples of biomarkers of developmental toxicity include low birth weight, chromosome anomalies, and delayed growth of specific organ systems, mental retardation, and subtle behavioural changes\textsuperscript{23}.

**BIOMARKERS OF NEUROTOXICITY**

The functions of the nervous system are complex and biomarkers may range from effects of chemicals on neural cellular and molecular processes to neurophysiological and neuro-behavioural measurements of complex functional entities. Inhibition of plasma and erythrocyte acetylcholine esterase (AchE) provides biomarkers of exposure to organophosphorus compounds and other cholinesterase inhibitors. While erythrocyte cholinesterase is similar to brain cholinesterase, and is therefore an effect biomarker, whereas plasma nonspecific pseudocholinesterase only reflects exposure and is not a marker of CNS effects. Assessment of peripheral nervous system dysfunction associated with exposure to chemicals can be carried out using electroneuromyography at the preclinical stage\textsuperscript{24}. Some well-established neurophysiological (e.g., evoked potentials, electroencephalography) and neurobehavioural\textsuperscript{25} measures may be used as biomarkers to evaluate CNS dysfunction induced by neurotoxicants. These tests must be carried out under well-controlled conditions. Methods for assessing changes in higher cognitive function (e.g., learning and memory) have been used extensively, e.g., in workers exposed to solvents or heavy metals, but require further refinement.

Available neuroimaging procedures, e.g., computed axial tomography (CAT), magnetic resonance imaging (MRI), nuclear magnetic resonance spectroscopy (MRS) and positron-emission tomography (PET), are considered non-invasive, but some of them require exposure to ionizing radiation. CAT and MRI can be carried out with current clinical techniques to assess chemically induced changes in the brain. The use of MRS and PET...
can provide a more detailed evaluation of the biochemical status (e.g., rate of energy generation, blood flow, L-glucose metabolism) in the central nervous system. They can be used as biomarkers for assessing exposure to neurotoxicants inducing brain alterations. Other promising biomarkers for neurotoxicity in animal studies include glial fibrillary acidic protein (localized in the astrocytes), which increases in localized areas within the brain where injury due to toxicants occurs

**BIOMARKERS FOR GENOTOXIC CARCINOGENS**

Various markers for genotoxic carcinogens are as follows:

**Protein adducts:** During the past few years, several monitoring studies have demonstrated the usefulness of protein adducts as biomarkers of exposure. Examples of chemicals that have been detected as protein adducts in human studies include ethylene and propylene oxide, aniline, cigarette smoke, aromatic amines such as 4-amino-biphenyl, and aflatoxin. Albumin adducts of aflatoxin B1 have also been used in epidemiological studies to determine their role in the etiology of hepatocellular carcinoma in man. A significant correlation was observed at the individual level between dietary intake and the level of albumin-bound aflatoxin in a chronically exposed population in the Gambia.

**Chromosome damage:** Both chromosome and chromatid aberrations are induced in individuals exposed to chemical mutagens. The chromosome aberrations are thought to arise from misrepair of lesions in the G0 stage of circulating lymphocytes as well as from precursor cells in bone marrow and thymus (Carrano and Natarajan, 1988). Chromatid aberrations include chromatid breaks, intrachanges and exchanges, while chromosome aberrations include acentric fragments, dicentric chromosomes and ring chromosomes. Balanced translocation and inversions can also arise and are difficult to quantify without banding analysis.

**Sister chromatid exchange:** Sister chromatid exchange (SCE) is considered to be a more sensitive, rapid and simple cytogenetic end-point than chromosome aberrations for
evaluating the genotoxic potential of a variety of mutagenic and carcinogenic agents. It is also used to detect and differentiate many chromosome fragility diseases that predispose to neoplasia. SCE is a DNA-replication-dependent phenomenon. Cellular factors such as nucleotide pools, repair and replication enzymes, and biorhythms can play an important role in its formation.

**Micronuclei:** Micronuclei are formed by condensation of acentric chromosomal fragments or by whole chromosomes that are left behind during anaphase movements (lagging chromosomes). The presence of micronuclei can therefore be taken as an indication of the previous existence of chromosomal aberrations.

**Aneuploidy:** Aneuploidy is a condition in which the number of chromosomes in cells of individuals is not an exact multiple of the typical haploid set for the species. Trisomy results when a single extra chromosome is added to a pair of homologous chromosomes. If one chromosome of a pair is missing, the result is monosomy. Absence of the pair is nullisomy. Two or more copies of a homologue result in tetrasomy or polysomy. Cells of individuals with missing or extra chromosomes are hypoploid or hyperploid. The best-known numerical abnormalities result in the syndromes of Down (trisomy of chromosome 21), Klinefelter (sex chromosome genotype is XXY), and Turner (sex chromosome genotype is X0). Aneuploidy is almost always found in human cancers.

**BIOMARKERS FOR NON-GENOTOXIC CARCINOGENESIS**

The biochemical indicators employed to detect the presence of cancers are collectively referred to as tumor markers. These are the abnormally produced molecules of tumor cells such as surface antigens, cytoplasmic proteins, enzymes and hormones. Tumor markers can be measured in serum or plasma. In theory, the tumor markers must ideally be useful for screening the population to detect the cancers. In practice, however, this has not been totally true. As such the tumor markers support the diagnosis of cancers, besides being useful for monitoring the response to therapy and for the early detection of recurrence.
### TABLE-2: SELECTED TUMOR MARKERS AND ASSOCIATED CANCERS

<table>
<thead>
<tr>
<th>TUMOR MAKER</th>
<th>ASSOCIATED CANCER (S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oncofetal antigens</td>
<td></td>
</tr>
<tr>
<td>Carcinoembryonic antigen (CEA)</td>
<td>Cancers of colon, stomach, lung, pancreas and breast</td>
</tr>
<tr>
<td>Alpha fetoprotein (AFP)</td>
<td>Cancer of liver and germ cells of testis</td>
</tr>
<tr>
<td>Cancer antigen – 125 (CA-125)</td>
<td>Ovarian cancer</td>
</tr>
<tr>
<td><strong>Hormones</strong></td>
<td></td>
</tr>
<tr>
<td>Human chorionic gonadotropin (hCG)</td>
<td>Choriocarcinoma</td>
</tr>
<tr>
<td>Calcitonin</td>
<td>Carcinoma of medially thyroid</td>
</tr>
<tr>
<td>Catecholamines and their metabolites (mainly vanillyl mandelic acid)</td>
<td>Pheochromocytoma and neuroblastoma</td>
</tr>
<tr>
<td><strong>Enzymes</strong></td>
<td></td>
</tr>
<tr>
<td>Prostatic acid phosphatase</td>
<td>Prostate cancer</td>
</tr>
<tr>
<td>Neuron specific enolase</td>
<td>Neuroblastoma</td>
</tr>
<tr>
<td><strong>Specific proteins</strong></td>
<td></td>
</tr>
<tr>
<td>Prostate specific antigen (PSA)</td>
<td>Prostate cancer</td>
</tr>
<tr>
<td>Immunoglobulins</td>
<td>Multiple myeloma</td>
</tr>
</tbody>
</table>

### TABLE-3: BIOMARKERS OF VARIOUS TOXICANTS

<table>
<thead>
<tr>
<th>METAL</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td>Measurement of blood δ aminolevulinic acid dehydratase (δ-ALA)</td>
</tr>
<tr>
<td></td>
<td>Free erythrocyte protoporphyrin are also useful in lab diagnosis</td>
</tr>
<tr>
<td></td>
<td>Increase in nucleated RBC in greater proportion then the degree</td>
</tr>
<tr>
<td></td>
<td>of anaemia.</td>
</tr>
<tr>
<td>Cu</td>
<td>Urine contain increased amount to hemoglobin &amp; bile pigment</td>
</tr>
<tr>
<td></td>
<td>Elevated bilirubin levels</td>
</tr>
<tr>
<td></td>
<td>Haemoglobinaemia</td>
</tr>
<tr>
<td></td>
<td>Haemoglobinuria</td>
</tr>
<tr>
<td></td>
<td>Elevated level of hepatic enzymes (AST), (LDH), (SDH) and Arginose</td>
</tr>
<tr>
<td>Cd</td>
<td>Urinary theronine and serine excretion are increased</td>
</tr>
<tr>
<td>Fe</td>
<td>Total serum iron levels are elevated</td>
</tr>
</tbody>
</table>
| NON-METALS | Serum hepatic enzymes & bilirubin levels are increased.  
|           | Haemoglobininurea. |

| Fluoride | Hyperkalaemia, hypocalcemia, hypomagnesaemia and hypoglycaemia.  
|          | Elevated fluorine level in plasma & urine. |

| Se       | Decreased fibrinogen & prothrombin activities.  
|          | Elevated serum ALT, AST & SDH levels.  
|          | Increased content of oxidized glutathione with a concomitant decrease of reduced glutathione. |

| P        | Hepatic enzymes & bilirubin levels elevated. |

| Chlorate | Estimation of met-Hb in blood. |

| PESTICIDES | Cholinesterase enzyme levels in blood & tissue (Brain). |

| AFLATOXINS | Serum enzymes ALT, AST, alkaline phosphates, ornithine, carbamoyltransferase and isocitric dehydrogenase are elevated. |

| AFLATOXINS | Serum bile acids are elevated.  
|           | Hyperbiliirubinaemia in chronic toxicosis. |

| OCCHRATOXINS | Elevated BUN and serum creatinine. |

| TETANUS | Tetanus toxin in serum. |

| POISONUS PLANT | Blood thiamine level decreased.  
|                | Blood pyruvate level increases. |

| OXALATE POISING | PCV decreases.  
|                 | BUN and blood potassium increases. |

| CARDIOTOXIC PLANT | Cardio glycosides in serum. |

| POISONUS ANIMALS | Stress related leucocytosis. |

| Honey bee | Increasing PCV, blood glucose, BUN, Potassium & calcium levels. |

| Toad      | Blood thiamine level decreased.  
|           | Blood pyruvate level increases. |

| Braken fern poisoning | Blood thiamine level decreased.  
|                      | Blood pyruvate level increases. |
Snake | Elevated plasma creatine kinase
---|---
CO | COHb in the blood
UREA POISONING | Elevated levels of blood glucose, blood lactate, BUN, aspartate aminotransferase, serum potassium
SALT POISONING | Plasma & CSF concentration of sodium
RADIATION | Absolute lymphocytes count

**CONCLUSION**

The use of biomarkers can improve the process for the assessment of health risks caused by exposure to chemicals. Biomarkers must be validated before application in the risk assessment process such as the relationship between the biomarker, the exposure, and the health outcome must be established.

The ethical, social and legal aspects of biomarkers require careful consideration prior to any application. These issues may impose constraints on research and use of biomarkers in risk assessment and risk management decisions.

Biomarkers speed up the process of diagnosing, monitoring and development of drug therapies. Early diagnosis will result in a higher percentage of successful patient treatment with the use of biomarkers. Discovery of new disease biomarkers and ability to measure them rapidly- preferably at initial point of care will revolutionize disease diagnosis.

**REFERENCES**


For Correspondence:
Anjana Panicker
Email: anu347@gmail.com